Supporting Information

Structure-Guided Design of Novel, Potent and Selective Macrocyclic Plasma Kallikrein Inhibitors

Zhe Li,[†,] James Partridge,[†] Abel Silva,[†] Peter Rademacher,[†] Andreas Betz,[†] Qing Xu,[†] Hing Sham,[†] Yunjin Hu,[‡] Yuqing Shan,[‡] Bin Liu,[‡] Ying Zhang,[‡] Haijuan Shi,[‡] Qiong Xu,[‡] Xubo Ma,[‡] Li Zhang[‡]*

[†] Global Blood Therapeutics, South San Francisco, CA 94080

[‡] Pharmaron Xi'an Co., Xi'an, Shaanxi, China 710018

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EXPERIMENTAL SECTION

Enzymatic Assays. Plasma Kallikrein and factor XIIa were purchased from Molecular Innovations (Novi MI), factor VIIA, factor Xa, factor XIa, α-thrombin and Lys-plasmin from Heamatologic Technology (Burlington Vermont), tPA from Innovative Research (Novi MI) and trypsin from Worthington Biochemical Corporation (Lakewood, NJ). The chromogenic substrates D-Pro-Phe-Arg-pNA (S-2302), N-Z-D-Arg-Gly-Arg-pNA (S-2765), D-Phe-Pip-Arg-pNA (S-2238) were from Aniara (Westchester, OH) or Diapharma (Detroit, respectively). Methylsulfonyl-D-Phe-Gly-Arg-pNA (Chromozyme t-PA) and Tosyl-Gly-Pro-Lys-4-nitranilide (Chromozyme PL) were purchased from Sigma (St. Louis, MI). Chromogenic substrates were prepared by dissolving 25 mg in 5mL of deionized water. Substrate concentration was calculated from absorbance using a molar extinction coefficient of 8270 $M^{-1}cm^{-1}$. All assays were performed in Assay Buffer (20 mM HEPES, 150 mM NaCl, 0.1% PEG 8000, 0.01% triton x-100, pH 7.4)

For the determination of IC_{50}, 8 μL of a serial dilution of inhibitor in DMSO including one blank were added to 72 μL of enzyme in Assay buffer in rows of a 96 well plate. After incubation for 30 min and addition of substrate, the initial rates of substrate hydrolysis determined by continuously monitoring the increase of absorbance at 405 nm in a kinetic plate reader Envision (Perkin-Elmer, Waltham, MA). The

dependence of the initial rates on the concentration of inhibitor was analyzed by regression to a sigmoid logistic function and the IC_{50} was interpolated. Seven increasing concentrations of inhibitor. Final concentration of chromogenic substrate for the individual enzymes tested was 200 μM, except for pKal which was 600 μM. All enzyme kinetic measurements were carried out at ambient temperature.

Protease	[Protease] (nM)	Substrate
fXa	0.5	S-2765
fXIa	0.5	S-2366
fXIIa	10	S-2302
t-PA	10	Chromozyme TPA
bovine trypsin	0.2	S-2765
Lys-plasmin	3	Chromozyme PL
thrombin	0.5	S-2238
pKal	0.25	S-2302
C1s protease	10	S-2288

***In-vivo* Studies**. Test compounds were subjected to pharmacokinetic studies on male Sprague-Dawley rats with three animals in each group. Compounds administered by oral gavage as a suspension in 0.5% methylcellulose (20 mg/kg). Blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h following oral dosing and the blood was centrifuged to separate the plasma. The concentration of test compounds were determined by high pressure liquid chromatography/tandem mass spectrometry (LC-MS/MS). Relevant pharmacokinetic parameters were derived by noncompartmental analysis (Phoenix version 6.3.0.395). *In vivo* studies were carried out at Portola Pharmaceuticals, Inc. (South San Francisco, CA 94080). All procedures were approved and carried out in accordance with Portola Inc.'s Institutional Animal Care and Use Committee protocol PPI-009-15.

***In-vitro* Microsomal Stability Studies**. Procedures and analysis for human and rat microsomal studies. In a 96-deep well plate 200 μL of 0.5 M Potassium phosphate pH 7.4 is added to 648 μL of water along with 50 μL of either HLM's or RLM's (1mg/ml final concentration) and 2 μL of a 2 mM solution of test compound dissolved in DMSO. The plate was incubated at 37 ºC for 5 min followed by the addition of 100 μL of 10 mM NADPH solution in H_2O. The total incubation volume is 1 ml. 50 μL aliquots are removed and added to 100 μL of ACN (containing 0.1% formic acid and internal standard) at 0, 5, 10, 15, 30, 45, and 60 min. At the conclusion of the experiment the plate is centrifuged at 4000 RPM for 5 min, the supernatant removed and samples analyzed by LC/MS/MS.

Table 3. Measured permeability across MCDK cells for compounds **2**, **29a**, **29b** and **30**.

Cmpd	Concentration (μM)	$P_{app,\ A-B}$ ($x10^{-6}$ cm/s)		Recover Rate (%)
		Value	Mean	
Digoxin	5	1.3	1.1	97
		0.9		
Propranolol	5	58.3	56.0	97
		53.7		
2	1	0.973	0.996	81
		1.086		
		0.928		
30	1	0.55	0.43	82
		0.32		
29	1	0.28	0.34	79
		0.40		
29a	1	0.30	0.26	80
		0.21		
29b	1	0.67	0.62	77

Chemistry/Compound Characterization: Reagents and solvents were purchased from Aldrich Chemical, Acros Organics, Alfa Aesar, AK Scientific, TCI America, Shanghai BePharm Ltd, J&K Scientific Ltd and used as received unless otherwise indicated. Air- and/or moisture-sensitive reactions were carried out under a nitrogen or argon atmosphere in oven-dried glassware using anhydrous solvents from Pharmaron. Air- and/or moisture-sensitive reagents were transferred via syringe or cannula, and introduced into reaction vessels through rubber septa. Solvent removal was accomplished with a rotary evaporator at ~10–50 Torr. Microwave irradiation was carried out with a Biotage initiator system. Automated silica gel column chromatography was carried out using a Biotage SP1 system and silica gel cartridges from Biotage. Analytical TLC plates from Merk (Silica Gel 60 F_{254}) were employed for TLC analyses. ^{1}H NMR spectra were recorded on a Bruker Avance III 300 MHz. Chemical shifts are reported in δ units (ppm) relative to TMS as an internal standard. Coupling constants (*J*) are reported in

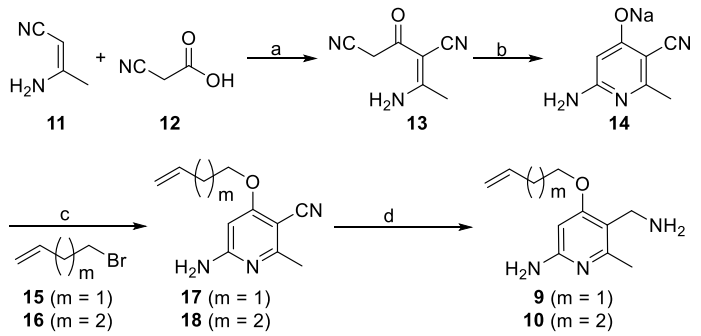
hertz (Hz). Characterization data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, br=broad, m=multiplet), coupling constants, number of protons, mass to charge ratio.

All analogs submitted for testing were judged to be of $\geq 95\%$ purity based on analytical LC/MS analysis performed on a Shimadzu LCMS-2020 Series, a quadruple mass spectrometer equipped with a Phenomenex Kinetex XB-C_{18} column (50 × 3.0 mm, 2.6 μm), at 40 degree C using a mobile phase of water-acetonitrile containing 0.05% TFAwith a flow rate of 1.5 mL/min. Gradient elution was employed wherein the acetonitrile: water ratio was increased linearly from 5% to 100% acetonitrile over 2 min, then maintained at 100% acetonitrile for 0.8 min, and then decreased to 5% acetonitrile over 0.1 min, and maintained at 5% acetonitrile for 0.1 min. Compound purity was determined by integrating peak areas of the liquid chromatogram, monitored at 254 nm.

General Procedure A for Amide Coupling with 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) for the Preparation of 4, 5, 6 and 39. To a solution of appropriate carboxylic acid (0.5 to 1.0 mmol), appropriate amine (0.5 to 1.0 mmol), and HATU (1.2 equiv.) in anhydrous DMF (4 to 8 mL), diisopropylethylamine (DIPEA) (2.5 equiv.) was added. The reaction mixture was stirred at rt for 0.5 to 12 h. The mixture was diluted with EtOAc (50 mL), washed with brine (3 x 20 mL) and concentrated to give the crude product, which was purified by silica gel column chromatography (dichloromethane/methanol: 8:1) to give the corresponding amides in good yield.

General Procedure B for Grubbs Ring Closing Metathesis for the Preparation of 3, 29, 30, and 32. The reaction vial containing a solution of bis-alkene (0.10 mmol, 1.0 eq) in DCE (20 mL) was purged and maintained under an inert atmosphere of nitrogen. The 2nd generation Grubbs catalyst (20 mg, 0.024 mmol, 0.24 equiv) was added. The resulting mixture was heated and stirred at 70^{o}C for 2 - 48 h under nitrogen. The mixture was then concentrated and the resulting residue was purified by silica gel column chromatography (DCM/MeOH, 10:1) or by prep-TLC (DCM/MeOH,5:1) to afford the desired macrocyclic analogs in low to moderate yields (4 - 64% yield) as a mixture of cis- and trans-isomers. Further HPLC purification may be needed to separate the olefinic isomers using the following conditions: (1) Waters 5 μM XBridge C18 column, 19 × 150 mm; (2) elution gradient: 30% to 45% MeCN in water over 8 min run time, where the aqueous phase contains 10 mM NH_4HCO_3 and 0.05% ammonia.

Scheme 1. Synthesis of Aminopyridine Intermediates 9 and 10[a]



[a]Reagents and conditions: (a) Ac_2O, dioxane, reflux 2 h, 44%; (b) EtONa, EtOH, reflux, 89%; (c) for **17** K_2CO_3, 4-bromobut-1-ene (**15**), DMF, 50 °C, 16 h, 74%; for **18** K_2CO_3, 5-bromopent-1-ene (**16**), DMF, 50 °C, 16 h, 100%; (d) LAH, Et_2O, reflux, 16 h, 46% for **9** and 38% for **10**.

(2Z)-2-(1-aminoethylidene)-3-oxopentanedinitrile (**13**). Acetic anhydride (44.8 g, 438.5mmol, 1.20 equiv) was added dropwise over 20 min to a solution of 2-cyanoacetic acid (37.1 g, 436.2 mmol, 1.20 equiv) and (2Z)-3-aminobut-2-enenitrile (30.0 g, 365.4 mmol, 1.00 equiv) in dioxane (300 mL). The mixture was heated at 100 °C for 2 h and cooled to rt. Yellow crystals were formed, which were collected by filtration to afford the title compound as a yellow solid (23.2 g, 44%). LCMS (ES) *m/z* 150.2 $[M+1]^+$.

6-Amino-4-hydroxy-2-methylnicotinonitrile (14). Compound **13** (23.0 g, 154.2 mmol, 1.00 equiv) was added to a 500 mL round-bottom flask containing sodium ethoxide (11.0 g, 161.6 mmol, 1.05 equiv) in anhydrous ethanol (300 mL). The resulting mixture was heated at 80 °C for 1 h and then cooled to rt. Yellow crystals were formed, which were collected by filtration to provide the sodium salt of compound **14** as a yellow solid (20.5 g, 89%). LCMS (ES) *m/z* 150.2 $[M+1]^+$.

6-Amino-4-(but-3-en-1-yloxy)-2-methylnicotinonitrile (17). To a solution of sodium 6-amino-3-cyano-2-methylpyridin-4-olate (**14**, 3.42 g, 20.0 mmol, 1.00 equiv) in DMF (30 mL) was added K_2CO_3 (2.76 g, 20.0 mmol, 1.00 equiv) and 4-bromobut-1-ene (**15**, 2.97 g, 22.0 mmol, 1.10 equiv). The reaction mixture was heated and stirred at 50 °C for 16 h. Excess solvents were removed under vacuum at 50 °C on a rotary evaporator. The resulting residue was diluted with EtOAc (100 mL), washed with brine (2 x 20 mL), dried over anhydrous sodium sulfate. Organic layer was concentrated under vacuum to give a gray solid, which was then triturated with TBME (2 x 30 mL) to provide the title compound as a gray solid (3.01 g, 74%). LCMS (ES) *m/z* 204.2 $[M+1]^+$.

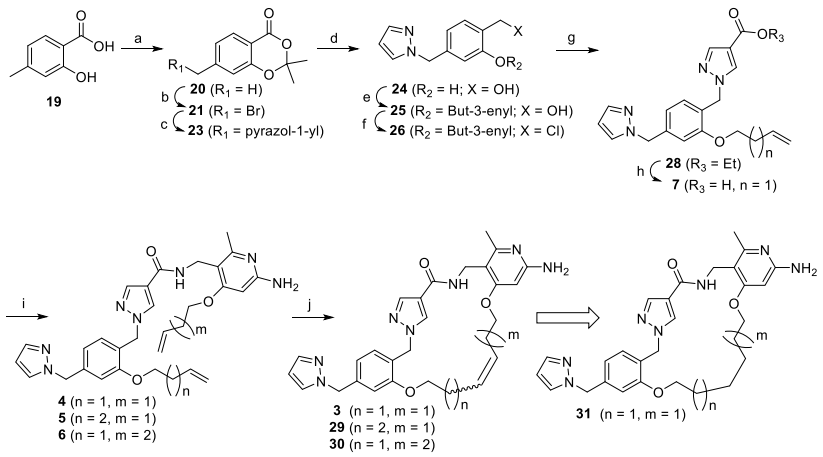
5-(Aminomethyl)-4-(but-3-enyloxy)-6-methylpyridin-2-amine (9). To a solution of 6-amino-4-(but-3-enyloxy)-2-methylnicotinonitrile (**17**, 3.00 g, 14.8 mmol, 1.00 equiv) in ether (100 mL) in a 250-mL round-bottom flask over ice bath was added LAH (5.46 g, 148 mmol, 10.00 equiv) in portions over 20 min. The ice bath was removed and the reaction mixture was heated and stirred at 40 °C for 16 h

under nitrogen atmosphere. The reaction was cooled to 0 °C and diluted with ether (100 mL), water (5.5 mL), aqueous sodium hydroxide (15%, 11.0 mL), and water (16.5 mL) was added sequentially. The solids were filtered and washed with ether (3 x 30 mL). Solvents were removed from the filtrate on a rotary evaporator. The resulting residue was purified on a silica column with DCM/MeOH (10/1) to provide the title compound as a yellow solid (1.40 g, 46%). LCMS (ES) *m/z* 208.2 $[M+1]^+$.

6-amino-2-methyl-4-(pent-4-enyloxy)nicotinonitrile (18). To a solution of sodium 6-amino-3-cyano-2-methylpyridin-4-olate (**14**, 1.03 g, 6.0 mmol, 1.00 equiv) in DMF (10.0 mL) was added K_2CO_3 (0.83 g, 6.0 mmol, 1.00 equiv) and 5-bromopent-1-ene (**16**, 0.98 g, 6.6 mmol, 1.10 equiv). The resulting mixture was stirred at 50 °C for 16 h. After cooling to rt, the mixture was diluted with EtOAc (100 mL), washed with brine (4 x 20 mL), dried over anhydrous sodium sulfate and concentrated under vacuum to give the crude product as a brown solid, which was triturated with TBME (2 x 10 mL) to provide 1.30 g (100%) of the title compound as a brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.81 (s, 2H), 5.90-5.81 (m, 2H), 5.08-4.97 (m, 2H), 4.01 (t, *J*=6.3 Hz, 2H), 2.32 (s, 3H), 2.19-2.14 (m, 2H), 1.84-1.79 (m, 2H). LCMS (ES) *m/z* 218.2 $[M+1]^+$.

5-(Aminomethyl)-6-methyl-4-(pent-4-enyloxy)pyridin-2-amine (10). To a cold solution of 6-amino-2-methyl-4-(pent-4-en-1-yloxy)pyridine-3-carbonitrile (**18**, 1.30 g, 5.98 mmol, 1.00 equiv) in ether (30 mL) over ice-bath was added LAH (2.30 g, 60.61 mmol, 10.00 equiv) in portions. After the addition, the ice-bath was removed and the reaction was heated and stirred at 45 °C for 48 h under N_2. The reaction mixture was diluted with ether (50 mL), quenched with water (9.2 mL) and 15% aqueous solution of sodium hydroxide (2.3 mL). The mixture was filtered and filtrate was concentrated on a rotary evaporator to afford 5-(aminomethyl)-6-methyl-4-(pent-4-en-1-yloxy) pyridin-2-amine as a yellow oil (500 mg, 38%). LCMS (ES) *m/z* 222.2 $[M+1]^+$.

Scheme 2. Synthesis of Macrocycles 3, 29, 30 and 31[a]



[a]Reagents and conditions: (a) TFAA,TFA, acetone, rt, 3 d, 46%; (b) NBS, BPO, CCl_4, 75 °C, 1 h, 66%; (c) pyrazole **22**, K_2CO_3, 50 °C, 2 h, 100%; (d) LAH, THF, -78 °C, 1 h, 84%; (e) **15**, Na_2CO_3, DMF, 80 °C, 14 h, 30%; (f) $SOCl_2$, DCM, rt, 30 min, 93%; (g) ethyl 1H-pyrazole-4-carboxylate **27**, K_2CO_3, DMF, rt, 14 h, 65%; (h)NaOH, MeOH/H_2O, rt, 85%; (i) **9** or **10**, HATU, DIPEA, DMF, rt, 14 h, 63% for **4**; 59% for **5**; (j) Grubbs' catalyst (II), DCE, 70 °C, 12 to 48 h; 4% for **3**; 13% for **29**.

2,2,7-Trimethyl-4H-benzo[d][1,3]dioxin-4-one (20). A mixture of 2-hydroxy-4-methylbenzoic acid (**19**, 10.0 g, 65.73 mmol, 1.00 equiv), TFA (50 mL), TFAA (30 mL) and acetone (7.6 g, 130.85 mmol, 2.00 equiv) was stirred at rt for 3 days. The mixture was diluted with H_2O (150 mL) and extracted with EtOAc (3 x 150 mL). The combined organic layers were concentrated, and the resulting residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 1:10). This gave 2,2,7-trimethyl-2,4-dihydro-1,3-benzodioxin-4-one as a yellow solid (5.8 g, 46%). LCMS (ESI) *m/z* 193 $[M+H]^+$.

7-(bromomethyl)-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (21). Benzoyl peroxide (BPO) (621 mg, 2.42 mmol, 0.22 equiv) was added to a mixture of 2,2,7-trimethyl-2,4-dihydro-1,3-benzodioxin-4-one (**20**, 2.24 g, 11.65 mmol, 1.00 equiv) and NBS (2.41 g, 13.54 mmol, 1.20 equiv) in CCl_4 (40 mL). The resulting solution was heated and stirred at 75 °C for 1 h. It was concentrated on a rotary evaporator and the resulting residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 1:10). This resulted in 2.10 g (66%) of 7-(bromomethyl)-2,2-dimethyl-2,4-dihydro-1,3-benzodioxin-4-one as a white solid. LCMS (ESI) *m/z* 271 $[M+H]^+$. t_R: 1.57 min.

7-((1H-pyrazol-1-yl)methyl)-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (23). A suspension of 7-(bromomethyl)-2,2-dimethyl-2,4-dihydro-1,3-benzodioxin-4-one (**21**, 2.10 g, 7.75 mmol, 1.00 equiv), potassium carbonate (1.10 g, 7.96 mmol, 1.03 equiv), and 1H-pyrazole (**22**, 635 mg, 9.33 mmol, 1.20 equiv) in DMF(40 mL) was heated and stirred at 50 °C for 2h. The mixture was concentrated on a rotary evaporator, the resulting residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 1:3). This resulted in 2.00 g (100%) of 2,2-dimethyl-7-(1H-pyrazol-1-ylmethyl)-2,4-dihydro-1,3-benzodioxin-4-one as a yellow solid. LCMS (ESI) *m/z* 259 $[M+H]^+$.

5-((1H-pyrazol-1-yl)methyl)-2-(hydroxymethyl)phenol (24). To a solution of 2,2-dimethyl-7-(1H-pyrazol-1-ylmethyl)-2,4-dihydro-1,3-benzodioxin-4-one (**23**, 1.80 g, 6.97 mmol, 1.00 equiv) in THF (18 mL) at -78 °C was added LAH (318 mg, 8.38 mmol, 1.20 equiv) in portions over 10 min. The mixture was stirred at -78 °C for 1 h and warmed to 0 °C. The reaction mixture was quenched by the addition of water (0.4 mL), acidified with 1N aqueous HCl until a clear solution was obtained. It was then diluted with EtOAc (100 mL), washed with brine (3 x 30 mL). The organic layers were dried over anhydrous sodium sulfate and concentrated on a rotary evaporator to afford a yellow residue, which was purified

by silica gel column chromatography (ethyl acetate/petroleum ether, 10:1) to give 1.20 g (84%) of 2-(hydroxymethyl)-5-(1H-pyrazol-1-ylmethyl)phenol as a yellow solid. ^{1}H NMR (300MHz, DMSO, *ppm*): δ 9.35 (s, 1H), 7.75 (d, *J*=1.8 Hz, 1H), 7.44 (d, *J*=1.5 Hz, 1H), 7.21 (d, *J*=7.8 Hz, 1H), 6.65 (d, *J*=7.5 Hz, 1H), 6.59 (d, *J*=1.5 Hz, 1H), 6.26 (t, *J*=2.1 Hz, 1H), 5.21 (s, 2H), 4.91 (t, *J*=5.7 Hz, 1H), 4.43 (d, *J*=5.7 Hz, 2H). LCMS (ESI) *m/z* 205 $[M+H]^+$.

(4-((1H-pyrazol-1-yl)methyl)-2-(but-3-enyloxy)phenyl)methanol (25). A mixture of 2-(hydroxymethyl)-5-(1H-pyrazol-1-ylmethyl)phenol (**24**, 1.0 g, 4.90 mmol, 1.00 equiv), 4-bromobut-1-ene (**15**, 788 mg, 5.84 mmol, 1.20 equiv), and sodium carbonate (1.04 g, 9.81 mmol, 2.00 equiv) in DMF(15 mL) was heated to 80^{o}C and stirred for 14 h. The mixture was diluted with water (50 mL) and extracted with EtOAc (3 x 100 mL). The organic combined organic layers were dried over anhydrous sodium sulfate and concentrated on a rotary evaporator to dryness. The resulting yellow residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 1:1) to afford 380 mg (30%) of [2-(but-3-en-1-yloxy)-4-(1H-pyrazol-1-ylmethyl)phenyl]methanol as a yellow oil. ^{1}H NMR (300MHz, $CDCl_3$, *ppm*): δ 7.61 (s, 1H), 7.42 (d, *J*=2.1 Hz, 1H), 7.25 (d, *J*=7.5 Hz, 1H), 6.83 (d, *J*=7.2 Hz, 1H), 6.79 (s, 1H), 6.33 (s, 1H), 5.95-5.82 (m, 1H), 5.36 (s, 2H), 5.24-5.14 (m, 2H), 4.66 (s, 2H), 4.06 (t, *J*=6.3 Hz, 2H), 2.60-2.54 (m, 2H); LCMS (ESI) *m/z* 259 $[M+H]^+$.

1-(3-(but-3-enyloxy)-4-(chloromethyl)benzyl)-1H-pyrazole (26). To a solution of [2-(but-3-en-1-yloxy)-4-(1H-pyrazol-1-ylmethyl)phenyl]methanol (**25**, 380 mg, 1.47 mmol, 1.00 equiv) in dichloromethane (10 mL) was added thionyl chloride (351 mg, 2.95 mmmol, 2.00 equiv) over a period of 10 min. The resulting solution was stirred for 30 min at rt and then concentrated. This resulted in 380 mg (93%) of 1-[[3-(but-3-en-1-yloxy)-4-(chloromethyl)phenyl]methyl]-1H-pyrazole as a yellow oil. LCMS (ESI) *m/z* 277 $[M+H]^+$.

Ethyl 1-(4-((1H-pyrazol-1-yl)methyl)-2-(but-3-enyloxy)benzyl)-1H-pyrazole-4-carboxylate (28). A mixture of 1-[[3-(but-3-en-1-yloxy)-4-(chloromethyl)phenyl]methyl] -1H-pyrazole (**26**, 400 mg, 1.45 mmol, 1.00 equiv), ethyl 1H-pyrazole-4-carboxylate (**27**, 244 mg, 1.74 mmol, 1.20 equiv), and K_2CO_3 (400 mg, 2.90 mmol, 2.00 equiv) in DMF (18 mL) was stirred overnight at rt. Then it was diluted with water (60 mL) and extracted with EtOAc (3 x 60 mL). The organic layers were combined, dried over anhydrous sodium sulfate and concentrated. The resulting residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 1:3) to give 360 mg (65%) of ethyl 1-[[2-(but-3-en-1-yloxy)-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxylate as a yellow oil. LCMS (ESI) *m/z* 381 $[M+H]^+$. t_R: 1.94 min.

1-(4-((1H-pyrazol-1-yl)methyl)-2-(but-3-enyloxy)benzyl)-1H-pyrazole-4-carboxylic acid (7). To a solution of ethyl 1-[[2-(but-3-en-1-yloxy)-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxylate (**28**, 380 mg, 1.00 mmol, 1.00 equiv) in methanol (8 mL) was NaOH (80 mg, 2.00 mmol, 2.00 equiv) in water (2 mL). The resulting solution was stirred overnight at rt. The mixture was concentrated on a rotary evaporator to dryness, diluted with H_2O (10 mL), and adjusted to pH ~ 4 by the addition of 2N aqueous HCl. The mixture was extracted with EtOAc (3 x 30 mL), combined organic layers were dried over sodium sulfate and concentrated to afford 300 mg (85%) of 1-[[2-(but-3-en-1-yloxy)-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxylic acid as a white solid.

1-(4-((1H-pyrazol-1-yl)methyl)-2-(but-3-enyloxy)benzyl)-N-((6-amino-4-(but-3-enyloxy)-2-methylpyridin-3-yl)methyl)-1H-pyrazole-4-carboxamide (4). The title compound was prepared from acid **7** and amine **9** according to the general procedure of amide coupling with HATU (Method A) (yellow solid, 63%). ^{1}H NMR (300MHz, DMSO-d_6, *ppm*): δ 8.07 (s, 1H), 7.82 (s, 1H), 7.80 (d, *J*=1.8 Hz, 1H), 7.63 (t, *J*=4.2 Hz, 1H), 7.44 (d, *J*=1.2 Hz, 1H), 6.99 (d, *J*=7.8 Hz, 1H), 6.91 (s, 1H), 6.72 (d, *J*=7.8 Hz, 1H), 6.25 (t, *J*=2.1Hz, 1H), 5.88-5.80 (m, 3H), 5.70 (s, 2H), 5.28 (s, 2H), 5.19 (s, 2H), 5.16-4.96 (m, 4H), 4.22 (d, *J*=4.2 Hz, 2H), 3.99-3.90 (m, 4H), 2.50-2.40 (m, 4H), 2.19 (s, 3H). LCMS (ESI) *m/z* 542 $[M+H]^+$.

19-amino-21-methyl-6-(1H-pyrazol-1-ylmethyl)-9,16-dioxa-1,20,24,28-tetraazatetracyclo[24.2.1.0^{3},^{8}.0^{17},^{22}]nonacosa-3,5,7,12,17(22),18,20,26(29),27-nonaen-25-one (3). The title compound was prepared from **4** according to the general procedure of Grubbs RCM (Method B) (crude, black oil, 100%). LC-MS (ESI) *m/z* 514 $[M+H]^+$.

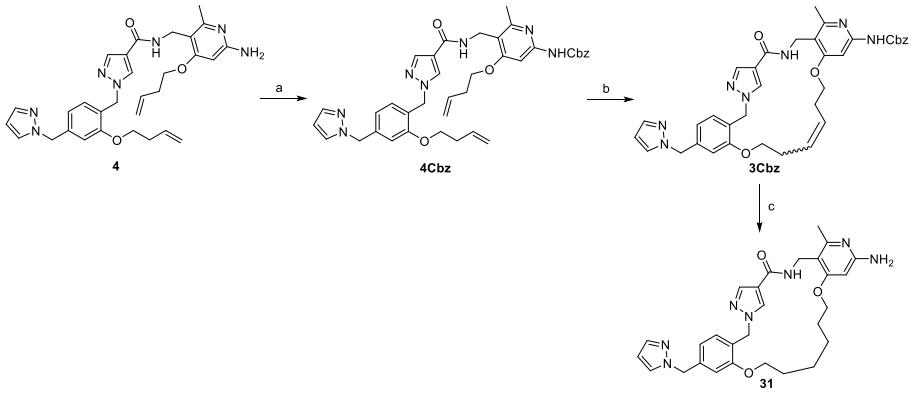
Separation of **3** into **3a** and **3b**: (**12E or 12Z)-19-amino-21-methyl-6-(1H-pyrazol-1-ylmethyl)-9,16-dioxa-1,20,24,28-tetraazatetracyclo[24.2.1.0^{3},^{8}.0^{17},^{22}]nonacosa-3,5,7,12,17(22),18,20,26(29),27-nonaen-25-one (cmpd 3a) and (12Z or 12E)- 19-amino-21-methyl-6-(1H-pyrazol-1-ylmethyl)-9,16-dioxa-1,20,24,28-tetraazatetracyclo[24.2.1.0^{3},^{8}.0^{17},^{22}]nonacosa-3,5,7,12,17(22),18,20,26(29),27-nonaen-25-one (cmpd 3b).** The above crude product **3** was purified by preparative HPLC with the following conditions. (1) Waters 5 µM XBridge C18 column, 19 × 150 mm; (2) elution gradient: 30% to 45% MeCN in water over 8 min run time, where the aqueous phase contains 10 mM NH_4HCO_3 and 0.05% ammonia. This provided 2.0 mg (4%) of **3a** as a white solid and 1.4mg (2%) of **3b**, respectively.

For **3a,** LCMS (ESI) *m/z* 514 $[M+H]^+$; t_R: 1.51 min; HRMS calcd for $C_{28}H_{32}N_7O_3$ (MH^+) 514.2567, found 514.2566; ^{1}H NMR (300MHz, DMSO-d_6, *ppm*) δ 8.02 (s, 1H), 7.82 (d, *J*=1.8 Hz, 1H), 7.76 (s, 1H), 7.60 (s, 1H), 7.45 (s, 1H), 7.38 (d, *J*=8.1 Hz, 1H), 6.91 (s, 1H), 6.76-6.74 (m, 1H), 6.26 (t, *J*=1.8

Hz, 1H), 5.85 (s, 1H), 5.70 (s, 2H), 5.65-5.50 (m, 1H), 5.35-5.30 (m, 1H), 5.29 (s, 2H), 5.17 (s, 2H), 4.21 (d, *J*=3.3 Hz, 2H), 3.91-3.85 (m, 2H), 3.79-3.65 (m, 2H), 2.34-2.28 (m, 7H).

For **3b**, LCMS (ESI) *m/z* 514 $[M+H]^+$; t_R: 1.54 min. HRMS calcd for $C_{28}H_{32}N_7O_3$ (MH^+) 514.2567, found 514.2564; ^1H NMR (300MHz, DMSO-d_6, *ppm*) δ 8.05 (s, 1H), 7.81 (s, 1H), 7.67 (s, 1H), 7.50 (s, 1H), 7.45 (s, 1H), 7.38 (d, *J*=7.5 Hz, 1H), 6.91 (s, 1H), 6.76-6.71 (m, 1H), 6.26 (s, 1H), 5.89 (s, 1H), 5.69 (s, 2H), 5.55-5.45 (m, 1H), 5.35-5.30 (m, 1H), 5.29 (s, 2H), 5.16 (s, 2H), 4.18 (d, *J*=4.5 Hz, 2H), 3.89-3.86 (m, 4H), 2.40-2.34 (m, 4H), 2.30 (s, 3H).

Scheme 2-2. Synthesis of Macrocyclic Analog 31[a]



[a]Reagents and conditions: (a) Cbz-Cl, $NaHCO_3$, acetone/H_2O, rt, 5 h, 85%; (b) Grubbs' catalyst (II), DCE, 70 °C, 14 h, 87%; (c) 10%Pd/C, H_2, MeOH, rt, 14 h, 21%.

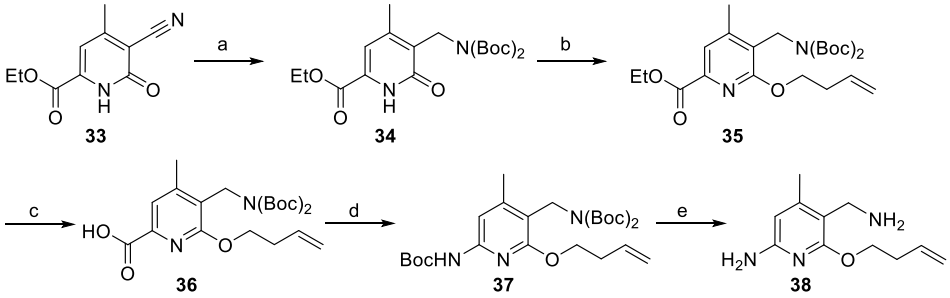
Benzyl (5-((1-(4-((1H-pyrazol-1-yl)methyl)-2-(but-3-en-1-yloxy)benzyl)-1H-pyrazole-4-carboxamido)methyl)-4-(but-3-en-1-yloxy)-6-methylpyridin-2-yl)carbamate (4Cbz). To a solution of N-[[6-amino-4-(but-3-en-1-yloxy)-2-methylpyridin-3-yl]methyl]-1-[[2-(but-3-en-1-yloxy)-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxamide (**4**, 30 mg, 0.06 mmol, 1.00 equiv) in a mixture of water (2 mL) and acetone (2 mL) was added sodium bicarbonate (9 mg, 0.11 mmol, 2.00 equiv) and Cbz-Cl (46 mg, 0.27 mmol, 5.00 equiv), respectively. The reaction mixture was stirred for 5 h at rt and then concentrated on a rotary evaporator. The resulting residue was purified by silica gel column chromatography (dichloromethane/methanol, 10:1) to afford 32 mg (85%) of benzyl N-[4-(but-3-en-1-yloxy)-5-[[(1-[[2-(but-3-en-1-yloxy)-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazol-4-yl)formamido]methyl]-6-methylpyridin-2-yl]carbamate as a yellow solid. LCMS (ESI) *m/z* 676.0 $[M+H]^+$; t_R: 2.07 min.

Benzyl ((3^{4}E)-14-((1H-pyrazol-1-yl)methyl)-7^2-methyl-4-oxo-3^{1}H-8,15-dioxa-5-aza-7(3,4)-pyridina-3(1,4)-pyrazola-1(1,2)-benzenacyclopentadecaphan-11-en-76-yl)carbamate (3Cbz). The

title compound was prepared from **4Cbz** according to the general procedure of Grubbs RCM (Method B) (yellow solid, 87%). LC-MS (ESI) *m/z* 648.0 $[M+H]^+$. t_R: 1.91 min.

19-Amino-21-methyl-6-(1H-pyrazol-1-ylmethyl)-9,16-dioxa-1,20,24,28-tetraazatetracyclo[24.2.1.0^{3},^{8}.0^{17},^{22}]nonacosa-3,5,7,17(22),18,20,26(29),27-octaen-25-one (31). To a solution of compound (**3Cbz**, 25 mg, 0.04 mmol, 1.00 equiv) in methanol (8 mL) was added 10% Pd/C (10 mg). The mixture was degassed and hydrogenated under one atmosphere of hydrogen overnight at rt. The reaction mixture was filtered and filtrate was concentrated to give an off-white residue, which was purified by prep-TLC to provide 4.1 mg (21%) of 19-amino-21-methyl-6-(1H-pyrazol-1-ylmethyl)-9,16-dioxa-1,20,24,28-tetraazatetracyclo[24.2.1.0³,8.0¹7,²²]nonacosa-3,5,7,17(22),18,20,26(29),27-octaen-25-one (**31**) as a white solid. LCMS (ESI) *m/z* 516 $[M+H]^+$; t_R: 1.90 min; HRMS calcd for $C_{28}H_{34}N_7O_3$ (MH^+) 516.2723, found 516.2522; ^{1}H NMR (300MHz, DMSO-d_6, *ppm*) δ 8.05 (s, 1H), 7.82 (s, 1H), 7.72 (s, 1H), 7.58 (t, *J*=4.2 Hz, 1H), 7.46 (s, 1H), 7.37 (d, *J*=8.1 Hz, 1H), 6.89 (s, 1H), 6.73 (d, *J*=7.8 Hz, 1H), 6.27 (s, 1H), 5.88 (s, 1H), 5.67 (s, 2H), 5.30 (s, 2H), 5.15 (s, 2H), 4.18 (d, *J*=4.2 Hz, 2H), 3.90-3.81 (m, 4H), 2.28 (s, 3H), 1.65-1.59 (m, 2H), 1.57-1.43 (m, 2H), 1.26-1.16 (m, 2H), 1.13-1.11 (m, 2H).

Scheme 3. Synthesis of Aminopyridine 38[a]



[a]Reagents and conditions: (a) Raney-Ni, H_2, Boc_2O, MeOH, rt, 16 h, 33%; (b) 4-bromobut-1-ene **15**, K_2CO_3, Ag_2CO_3, DMF, 80 °C, 16 h, 50%; (c) NaOH, THF, MeOH, H_2O, 50 °C, 16 h, 77%; (d) TEA, DPPA, t-BuOH, toluene, 100 °C, 16 h, 84%; (e) TFA, DCM, rt, 12 h, 98%.

Ethyl 5-((bis (tert-butoxycarbonyl) amino) methyl)-4-methyl-6-oxo-1, 6-dihydropyridine-2-carboxylate (34). To a solution of ethyl 5-cyano-4-methyl-6-oxo-1, 6-dihydropyridine-2-carboxylate (**33**, 8.00 g, 38.80 mmol, 1.00 equiv) in methanol (80 mL) was added Raney-Ni (4.0 g), and Boc_2O (16.93 g, 77.57 mmol, 2.00 equiv). The reaction vessel was allowed to go through three cycles of degassing and purging with hydrogen, and then kept at rt for 16 h with stirring. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 1:5) to give 5.3 g (33%) of

ethyl 5-((bis (tert-butoxycarbonyl) amino) methyl)-4-methyl-6-oxo-1,6 dihydropyridine-2-carboxylate as a white solid. LCMS (ESI) *m/z* 311 $[M+H\text{-}Boc]^+$; t_R: 1.28 min.

Ethyl 5-((bis (tert-butoxycarbonyl) amino) methyl)-6-(but-3-enyloxy)-4-methylpicolinate (35). To a suspension of ethyl 5-([[(tert-butoxy)carbonyl]amino]methyl)-4-methyl-6-oxo-1,6-dihydropyridine-2-carboxylate (**34**, 2.00 g, 6.44 mmol, 1.00 equiv), potassium carbonate (887 mg, 6.42 mmol, 1.00 equiv), and Ag_2CO_3 (1.78 g, 6.44 mmol, 1.00 equiv) in DMF (50 mL) was added 4-bromobut-1-ene (1.74 g, 12.89 mmol, 2.00 equiv). The resulting mixture was heated and stirred at 80 ^oC overnight. It was then concentrated, diluted with EtOAc (100 mL), washed with brine (3 x 30 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated on a rotary evaporator to afford a yellow residue, which was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 1:5) to afford 1.13 g (50%) of ethyl 5-((bis (tertbutoxycarbonyl) amino) methyl)-6-(but-3-enyloxy)-4-methylpicolinate as a light yellow oil. LCMS (ESI) *m/z* 465 $[M+H]^+$; t_R: 1.87 min.

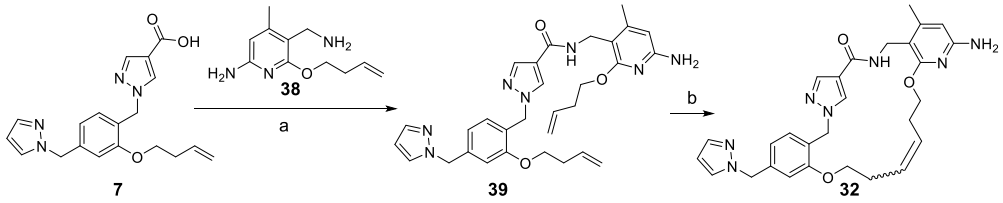
5-((Bis(tert-butoxycarbonyl)amino)methyl)-6-(but-3-enyloxy)-4-methylpicolinic acid (36). To a solution of ethyl 5-([bis[(tert-butoxy)carbonyl]amino]methyl)-6-(but-3-en-1-yloxy)-4-methylpyridine-2-carboxylate (**35**, 1.13 g, 2.43 mmol, 1.00 equiv) in a mixture of solvents (8 mL) (THF/methanol, 1:1) was added sodium hydroxide (107 mg, 2.68 mmol, 1.10 equiv) in water (2 mL). The reaction was heated and stirred at 50 ^oC for 16 h. Solvents removed under vacuum. The residue was diluted with water (30 mL), adjusted pH to 3.0 by the addition of 1N HCl aqueous solution, and extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated on a rotary evaporator to afford 818 mg (77%) of 5-((bis(tert-butoxycarbonyl)amino)methyl)-6-(but-3-enyloxy)-4-methylpicolinic acid as a light yellow oil. LCMS (ESI) *m/z* 437 $[M+H]^+$; t_R: 1.69 min.

Tert-butyl N-[[2-(but-3-en-1-yloxy)-6-[[(tert-butoxy) carbonyl] amino]pyridin-3-yl]methyl]-N-[(tert-butoxy)carbonyl]carbamate (37). To a solution of 5-([bis[(tert-butoxy)carbonyl]amino]methyl)-6-(but-3-en-1-yloxy)-4-methylpyridine-2-carboxylic acid (**36**, 1.60 g, 3.67 mmol, 1.00 equiv) in toluene (50 mL) was added sequentially tert-butanol (543 mg, 7.34 mmol, 2.00 equiv), TEA (1.11 g, 10.97 mmol, 3.00 equiv). The reaction was cooled to 0 ^oC and DPPA (1.21 g, 4.40 mmol, 1.20 equiv) added over 30 min. The reaction mixture was heated and kept at 100 ^oC for 16 h. After cooling to rt, the mixture was diluted with EtOAc (100 mL), washed with brine (2 x 50 mL), dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 1:5) to give 800 mg (44%) of tert-butyl N-[[2-(but-3-en-

1-yloxy)-6-[[(tert-butoxy)carbonyl]amino]pyridin-3-yl]methyl]-N-[(tert-butoxy)carbonyl]carbamate as a yellow oil. LCMS (ESI) *m/z* 408 $[M+H\text{-}Boc]^+$; t_R: 1.77 min.

***N*-(6-(but-3-enyloxy)-4-methyl-5-((2, 2, 2-trifluoroacetamido) methyl) pyridin-2-yl)-2, 2, 2-trifluoroacetamide (38).** To a solution of tert-butyl N-[[2-(but-3-en-1-yloxy)-6-[[(tert-butoxy) carbonyl]amino]-4-methylpyridin-3-yl]methyl]-N-[(tert-butoxy) carbonyl]carbamate (**37**, 770 mg, 1.52 mmol, 1.00 equiv.) in dichloromethane (10 mL) was added TFA(10 mL) dropwise over 10 min. The resulting solution was stirred for 12 h at rt. The mixture was concentrated under vacuum to give a sticky residue, which was dissolved in water (30 mL) and then lyophilized. This resulted in 613.7 mg (98%) of N-(6-(but-3-enyloxy)-4-methyl-5-((2,2,2-trifluoroacetamido)methyl)pyridin-2-yl)-2,2,2-trifluoroacetamide as a light brown solid. LCMS (ESI) *m/z* 191 $[M\text{-}NH_2]^+$; t_R: 0.99 min; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72 (br, 3H), 5.97-5.84（m, 2H), 5.17-5.06 (m, 2H), 4.23 (t, *J*=6.6 Hz, 2H), 3.82-3.80 (m, 2H), 2.55-2.34 (m, 2H), 2.18 (s, 3H).

Scheme 4. Synthesis of Macrocycle 32[a]



[a]Reagents and conditions: (a) HATU, DIPEA, DMF, rt, 14 h, 52%; (b) Grubbs' catalyst (II), DCE, 70 °C, 48 h, 63%.

N-[[6-amino-2-(but-3-en-1-yloxy)-4-methylpyridin-3-yl]methyl]-1-[[2-(but-3-en-1-yloxy)-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxamide (39). The title compound was prepared from acid **7** and amine **38** according to the general procedure of amide coupling with HATU (Method A) (yellow solid, 52%). LCMS (ESI) m/z 542 $[M+H]^+$; t_R: 1.90 min.

19-Amino-21-methyl-6-(1H-pyrazol-1-ylmethyl)-9,16-dioxa-1,18,24,28-tetraazatetracyclo[24.2.1.0^{3},^{8}.0^{17},^{22}]nonacosa-3,5,7,12,17(22),18,20,26(29),27-nonaen-25-one (cmpd 32). The title compound was prepared from **39** according to the general procedure of Grubbs RCM (Method B) (off-white solid, 63%) (mixture of two isomers). LCMS (ESI) m/z 514 $[M+H]^+$; t_R: 1.12 min.

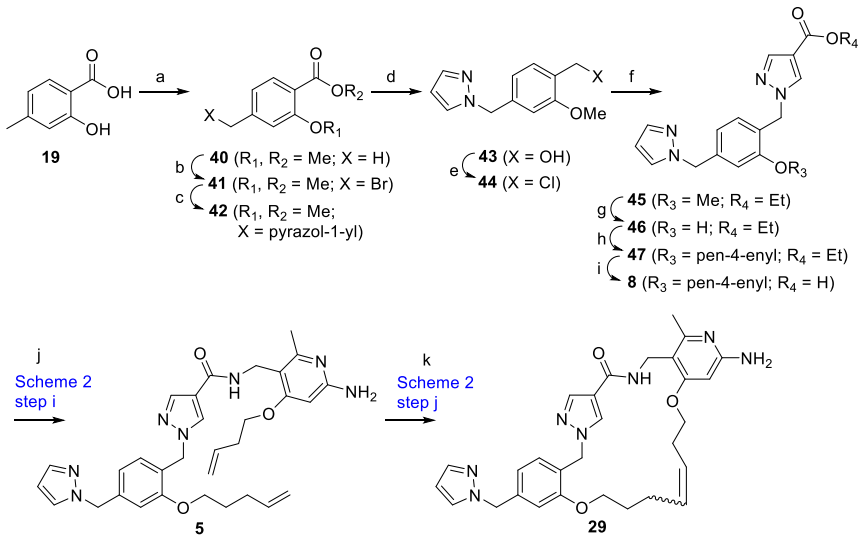
Separation of **32** into **32a** and **32b**: **(12E or 12Z)-19-amino-21-methyl-6-(1H-pyrazol-1-ylmethyl)-9,16-dioxa-1,18,24,28-tetraazatetracyclo[24.2.1.0^{3},^{8}.0^{17},^{22}]nonacosa-3,5,7,12,17(22),18,20,26(29),27-nonaen-25-one (cmpd 32a) and (12Z or 12E)-19-amino-21-methyl-6-(1H-pyrazol-1-ylmethyl)-9,16-dioxa-1,18,24,28-tetraazatetracyclo[24.2.1.0^{3},^{8}.0^{17},^{22}]nonacosa-3,5,7,12,17(22),18,20,26(29),27-**

nonaen-25-one (cmpd 32b). The isomeric mixture of **32** (90 mg) was separated by Prep-HPLC with the following conditions. (1) Waters 5 µM XBridge C18 column, 19 × 150 mm; (2) elution gradient: 30% to 45% MeCN in water over 8 min run time, where the aqueous phase contains 10 mM NH_4HCO_3 and 0.05% ammonia. This provided 9.0 mg (6%) of **32a** as an off-white solid and 10.1 mg (7%) of **32b** as an off-white solid.

For **32a**: LC-MS (ESI) *m/z* 514 $[M+H]^+$; t_R: 1.42 min; HRMS calcd for $C_{28}H_{32}N_7O_3$ (MH^+) 514.2567, found 514.2562; ^{1}H NMR (300 MHz，DMSO-d6): δ 8.05 (s, 1H), 7.82 (d, *J*=1.8 Hz, 1H), 7.66 (s, 1H), 7.50 (br, 1H), 7.45 (d, *J*=1.2 Hz, 1H), 7.37 (d, *J*=7.8 Hz, 1H), 6.91 (s, 1H), 6.73 (d, *J*=8.4 Hz, 1H), 6.26 (t, *J*=2.1 Hz, 1H), 5.82 (s, 1H), 5.66 (s, 2H), 5.60-5.40 (m, 1H), 5.38-5.22 (m, 3H), 5.15 (s, 2H), 4.18-4.09 (m, 4H), 3.86 (t, *J*=6.9 Hz, 2H), 2.50-2.34 (m, 4H), 2.18 (s, 3H).

For **32b**: LC-MS (ESI) *m/z* 514 $[M+H]^+$; t_R: 1.40 min; HRMS calcd for $C_{28}H_{32}N_7O_3$ (MH^+) 514.2567, found 514.2567; ^{1}H NMR (300 MHz，DMSO-d6): δ 8.06 (s, 1H), 7.82 (d, *J*=2.1 Hz, 1H), 7.73 (s, 1H), 7.55 (br, 1H), 7.45 (d, *J*=1.2 Hz, 1H), 7.37 (d, *J*=7.8 Hz, 1H), 6.90 (s, 1H), 6.76 (d, *J*=7.8 Hz, 1H), 6.26 (t, *J*=2.1 Hz , 1H), 5.84 (s, 1H), 5.66 (s, 2H), 5.60-5.45 (m, 1H), 5.38-5.25 (m, 3H), 5.16 (s, 2H), 4.21-4.09 (m, 4H), 3.73 (t, *J*=6.9 Hz, 2H), 2.30-2.19 (m, 2H), 2.17 (s, 3H), 2.05-2.00 (m, 2H).

Scheme 5. Synthesis of Macrocycle 29[a]



[a]Reagents and conditions: (a) CH_3I, K_2CO_3, DMF, rt, 16 h, 79%; (b) NBS, BPO, CCl_4, 80 °C, 16 h, 100%; (c) pyrazole **22**, K_2CO_3, 50 °C, 16 h, 21%; (d) LAH, THF, 0 - 25 °C, 1 h, 100%; (e) $SOCl_2$, DCM, 0 - 25 °C, 90 min, 100%; (f) ethyl 1H-pyrazole-4-carboxylate **27**, K_2CO_3, DMF, 50 °C, 14 h, 64%; (g) BBr_3, DCM, -78 - 0 °C, 2 h, 52%; (h) 5-bromopent-1-ene **16**, Cs_2CO_3, DMF; 90 °C, 2 h, 66%; (i) NaOH, MeOH/H_2O, 50 °C, 85%; (j) see scheme 2 step i, HATU, DIPEA, DMF, rt, 30 min, 59%; (k) see scheme 2 step j, Grubbs' catalyst (II), DCE, 70 °C, 12 h; 13%.

Methyl 2-methoxy-4-methylbenzoate (40). To a suspension of 2-hydroxy-4-methylbenzoic acid (**19**, 60.8 g, 399.61 mmol, 1.00 equiv) and potassium carbonate (165.6 g, 1.20 mol, 3.00 equiv) in DMF(800 mL) was CH_3I (98.9 mL, 1.6 mol, 4.00 equiv) dropwise with stirring at rt. The resulting mixture was stirred for 16 h at rt. It was then concentrated, diluted with MTBE (1000 mL), and washed with brine (3 x 300 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 1:10) to give 57.0 g (79 %) of methyl 2-methoxy-4-methylbenzoate as a light yellow oil. LCMS (ESI) *m/z* 181 $[M+H]^+$; ^1H NMR (300MHz, $CDCl_3$, *ppm*) δ 7.73-7.70 (m, 1H), 6.79-6.77 (m, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 2.37 (s, 3H).

Methyl 4-(bromomethyl)-2-methoxybenzoate (41). To a solution of methyl 2-methoxy-4-methylbenzoate (**40**, 57.0 g, 316 mmol, 1.00 equiv) and NBS (62.0 g, 348 mmol, 1.10 equiv) in CCl_4 (800 mL) was added BPO (4.05 g, 15.8 mmol, 0.05 equiv) in 5 portions over 10 min. The resulting mixture was heated and stirred at 80 ^oC for 16 h. After cooling to rt, the mixture was concentrated, the resulting residue was diluted with TBME (1000 mL) and washed with brine (3 x 500 mL). The organic layers were dried over anhydrous sodium sulfate and concentrated to give 86.9 g (100 %) of the title compound as a brown crude oil. LCMS (ESI) *m/z* 259 $[M+H]^+$.

Methyl 2-methoxy-4-(1H-pyrazol-1-ylmethyl)benzoate (42). A mixture of methyl 4-(bromomethyl)-2-hydroxybenzoate (**41**, 81.6 g, 316 mmol, 1.00 equiv), 1H-pyrazole (25.7 g, 379 mmol, 1.20 equiv), and potassium carbonate (43.6 g, 316 mmol, 1.00 equiv) in DMF (500 mL) was heated and stirred at 50 ^oC overnight. After concentration, the residue was diluted with EtOAc (1000 mL) and washed with brine (2 x 300 mL), dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel column chromatography (THF/petroleum ether, 1:1) to give the title compound (14.42 g, 20.8 %) of as a brown oil. LCMS (ESI) *m/z* 247 $[M+H]^+$.

[2-Methoxy-4-(1H-pyrazol-1-ylmethyl)phenyl]methanol (43). To a solution of methyl 2-methoxy-4-(1H-pyrazol-1-ylmethyl)benzoate (**42**, 14.42 g, 58.56 mmol, 1.00 equiv) in THF (150 mL) over ice bath was added $LiAlH_4$ (2.46 g, 64.82 mmol, 1.10 equiv) in portions over 20 min. The resulting mixture was stirred at 0 ^oC for 30 min and then warmed up to rt. Water (50 mL) and 1N aqueous NaOH were added until a clear solution was obtained. The mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over anhydrous sodium sulfate, and concentrated under vacuum. This resulted in 14.0 g (100 %) of [2-methoxy-4-(1H-pyrazol-1-ylmethyl)phenyl]methanol as white solid. LCMS (ESI) *m/z* 219 $[M+H]^+$.

1-[[4-(Chloromethyl)-3-methoxyphenyl]methyl]-1H-pyrazole (44). A solution of thionyl chloride (**43**, 9.44 g, 79.9 mmol, 2.00 equiv) in anhydrous DCM (50 mL) was added to a solution of [2-methoxy-4-(1H-pyrazol-1-ylmethyl)phenyl]methanol (8.72 g, 39.95 mmol, 1.00 equiv) in anhydrous DCM (150 mL) at 0 ^{o}C over 30 min. Ice bath was then removed and the reaction mixture was stirred at rt for 1.5 h. It was then concentrated to yield the title compound (10.33 g, 100 %) as a light yellow solid.

Ethyl 1-[[2-methoxy-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxylate (45). A suspension of 1-[[4-(chloromethyl)-3-methoxyphenyl]methyl]-1H-pyrazole (**44**, 10.33 g, 43.64 mmol, 1.00 equiv), ethyl 1H-pyrazole-4-carboxylate (**27**,7.96 g, 56.80 mmol, 1.30 equiv), and potassium carbonate (15.10 g, 109.1 mmol, 2.50 equiv) in DMF (200 mL) was heated and stirred at 50 ^{o}C overnight. After concentration, the residue was diluted with EtOAc (800 mL), washed with brine (3 x 200 mL), dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel column chromatography (dichloromethane/ethyl acetate, 1:1) to give the title compound (9.50 g, 64 %) as a light yellow solid. LCMS (ESI) *m/z* 341 $[M+H]^+$.

Ethyl 1-[[2-hydroxy-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxylate (46). To a cold solution of ethyl 1-[[2-methoxy-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxylate (**45**, 4.80 g, 14.1 mmol, 1.00 equiv) in dry DCM (100 mL) at -78 ^{o}C was added dropwise BBr_3 (42.3 mL, 1 mol/L in DCM, 42.3 mmol, 3.00 equiv) over 45 min. The reaction mixture was allowed to warm up to rt and stirred for 2 h. The mixture was poured into ice water (100 mL), extracted with DCM (3 x 200 mL). The combined organic layers were washed with brine (2 x 200 mL), dried over anhydrous sodium sulfate and concentrated under vacuum. This produced the product (2.41g, 52 %) as a light yellow solid**.** LCMS (ESI) *m/z* 327 $[M+H]^+$. ^{1}H NMR (300MHz, CD3OD, *ppm*) δ 8.32 (s, 1H), 8.22 (d, *J*=2.4 Hz, 1H), 8.16 (s, 1H), 7.88 (s, 1H), 7.17 (d, *J*=8.1 Hz, 1H), 6.78-6.71 (m, 3H), 5.58 (s, 2H), 5.35 (s, 2H), 4.28 (q, *J*=7.2 Hz, 2H), 1.33 (t, *J*=7.2 Hz, 3H).

Ethyl 1-[[2-hydroxy-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxylate (47). A mixture of ethyl 1-[[2-hydroxy-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxylate (**46**, 670 mg, 2.05 mmol, 1.00 equiv), 5-bromopent-1-ene (**16**, 605 mg, 4.06 mmol, 2.00 equiv) and Cs_2CO_3 (1.67 g, 5.13 mmol, 2.50 equiv) in DMF(10 mL) was heated and stirred at 90 ^{o}C for 2 h. After cooling to rt, the mixture was diluted with EtOAc (100 mL), washed with brine (4 x 30 mL), dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 1:3) to afford the title compound (537 mg, 66 %) as a light yellow solid. LCMS (ESI) *m/z* 395 $[M+H]^+$.

1-[[2-(pent-4-en-1-yloxy)-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxylic acid (8). To a solution of ethyl 1-[[2-(pent-4-en-1-yloxy)-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxylate (**47**, 537 mg, 1.36 mmol, 1.00 equiv) in methanol (8 mL) was added sodium hydroxide (108 mg, 2.70 mmol, 2.00 equiv) in water (4 mL). The resulting mixture was heated and stirred at 50 ^{o}C overnight. The reaction mixture was concentrated under vacuum to remove organic volatiles, water (10 mL) was then added, pH of the resulting solution was adjusted to 5-6 by the addition of 2 N HCl aqueous solution. The mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers was washed with brine (2 x 30 mL) and concentrated under vacuum. This gave the acid product (426 mg, 85 %) as a white solid. LC-MS (ESI) *m/z* 367 $[M+H]^+$.

N-[[6-amino-4-(but-3-en-1-yloxy)-2-methylpyridin-3-yl]methyl]-1-[[2-(pent-4-en-1-yloxy)-4-(1H-pyrazol-1-ylmethyl)phenyl]methyl]-1H-pyrazole-4-carboxamide (5). The title compound was prepared from acid **8** and amine **9** according to the general procedure of amide coupling with HATU (Method A) (yellow solid, 59%). LC-MS (ESI) *m/z* 556 $[M+H]^+$.

20-Amino-22-methyl-6-[(1H-pyrazol-1-yl)methyl]-9,17-dioxa-1,21,25,29-tetraazatetracyclo[25.2.1.0^{3},^{8}.0^{18},^{23}]triaconta-3,5,7,13,18(23),19,21,27(30),28-nonaen-26-one (cmpd 29). The title compound was prepared from **5** according to the general procedure of Grubbs RCM (Method B). The product (37.9 mg, 13 %) was obtained as a gray solid (mixture of cis- and transisomers). LCMS (ESI) *m/z* 528 $[M+H]^+$; t_R: 1.81 min; HRMS calcd for $C_{29}H_{34}N_7O_3$ (MH^+) 528.2723, found 528.2721; ^{1}H NMR (300MHz, DMSO-d_6, *ppm*) δ 7.97 (s, 1H), 7.84 (d, *J*=2.1 Hz, 1H), 7.72 (s, 1H), 7.69 (s, 1H), 7.46 (d, *J*=1.5 Hz, 1H), 7.39 (d, *J*=7.8 Hz, 1H), 6.85 (s, 1H), 6.72 (d, *J*=7.2 Hz, 1H), 6.48 (br, 2H), 6.27 (t, *J*=2.1 Hz, 1H), 6.01 (s, 1H), 5.40 (s, 2H), 5.34 (s, 2H), 5.15 (s, 2H), 4.19 (s, 2H), 3.95 (s, 2H), 3.81 (s, 2H), 2.40-2.31 (m, 5H), 1.60-1.56 (m, 4H); ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.84 (d, *J* = 0.7 Hz, 1H), 7.81 (d, *J* = 0.7 Hz, 1H), 7.73 – 7.66 (m, 1H), 7.52 – 7.46 (m, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 1.4 Hz, 1H), 6.79 (s, 1H), 6.31 (t, *J* = 2.2 Hz, 1H), 6.07 (s, 1H), 5.47 – 5.28 (m, 2H), 5.32 (s, 2H), 5.21 (s, 2H), 4.35 (s, 2H), 4.00 (t, *J* = 5.3 Hz, 2H), 3.85 (s, 2H), 2.40 (s, 5H), 1.67 – 1.57 (m, 4H); ^{13}C NMR (101 MHz, Methanol-d_4) δ 167.30, 163.32, 157.78, 157.61, 139.98, 139.11, 138.69, 131.92, 131.49, 130.31, 127.16, 121.65, 118.86, 117.22, 110.37, 110.01, 109.99, 105.56, 88.80, 68.96, 65.81, 54.72, 51.98, 34.09, 31.42, 28.27, 28.04, 17.66.

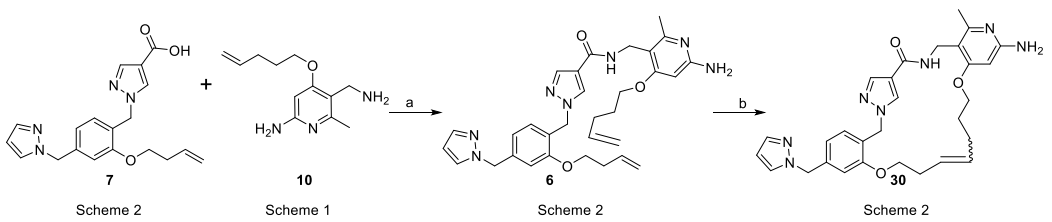
Two single olefinic isomers (**29a** and **29b**), (13E or 13Z)-20-amino-22-methyl-6-(1H-pyrazol-1-ylmethyl)-9,17-dioxa-1,21,25,29-tetraazatetracyclo[25.2.1.0^{3},^{8}.0^{18},^{23}]triaconta-3,5,7,13,18(23),19,21,27(30),28-nonaen-26-one and (13Z or 13E)-20-amino-22-methyl-6-(1H-pyrazol-1-ylmethyl)-9,17-dioxa-1,21,25,29-tetraazatetracyclo[25.2.1.0^{3},^{8}.0^{18},^{23}]triaconta-

3,5,7,13,18(23),19,21,27(30),28-nonaen-26-one were obtained after HPLC purification following conditions described in general procedure B. From 120 mg of **29**, 60.4 mg of **29a** and 3.5 mg of **29b** was recovered, respectively.

For **29a**: LCMS (ESI) *m/z* 528 $[M+H]^+$; t_R: 1.78 min; HRMS calcd for $C_{29}H_{34}N_7O_3$ (MH^+) 528.2723, found 528.2722; ^{1}H NMR (300MHz, DMSO-d_6): δ 7.98 (s, 1H), 7.84 (d, *J*=2.1 Hz, 1H), 7.72 (s, 1H), 7.62 (br, 1H), 7.46 (d, *J*=1.5 Hz, 1H), 7.40 (d, *J*=7.5 Hz, 1H), 6.85 (s, 1H), 6.72 (d, *J*=7.2 Hz, 1H), 6.30-6.27 (m, 3 H), 5.97 (s, 1H), 5.41 (s, 2H), 5.32 (s, 2H), 5.15 (s, 2H), 4.20 (d, *J*=4.2 Hz, 2H), 3.95 (br, 2H), 3.83 (br, 2H), 2.36-2.31 (m, 5H), 1.62-1.57 (m, 4H); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.80 (dd, *J* = 2.2, 0.7 Hz, 1H), 7.69 (d, *J* = 0.7 Hz, 1H), 7.57 (t, *J* = 4.3 Hz, 1H), 7.43 (dd, *J* = 1.8, 0.7 Hz, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 6.82 (d, *J* = 1.5 Hz, 1H), 6.70 (dd, *J* = 7.6, 1.4 Hz, 1H), 6.27 (m, 2H), 6.24 (t, *J* =2.0 Hz, 1H), 5.92 (s, 1H), 5.38 (td, *J* = 4.8, 3.1 Hz, 2H), 5.28 (s, 2H), 5.12 (s, 2H), 4.17 (d, *J* = 4.2 Hz, 2H), 3.90 (t, *J* = 5.3 Hz, 2H), 3.78 (t, *J* = 5.2 Hz, 2H), 2.31 (q, *J* = 5.2 Hz, 2H), 2.26 (s, 3H), 1.57 (ddt, *J* = 27.2, 9.7, 5.6 Hz, 4H);^{1}H NMR (400 MHz, Methanol-d_4) δ 7.84 (d, *J* = 0.8 Hz, 1H), 7.81 (d, *J* = 0.7 Hz, 1H), 7.69 (dd, *J* = 2.3, 0.7 Hz, 1H), 7.49 (dd, *J* = 1.9, 0.7 Hz, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 1.5 Hz, 1H), 6.79 (s, 1H), 6.31 (t, *J* = 2.2 Hz, 1H), 6.06 (s, 1H), 5.45 – 5.27 (m, 2H), 5.32 (s, 2H), 5.21 (s, 2H), 4.35 (s, 2H), 3.99 (t, *J* = 5.2 Hz, 2H), 3.85 (t, *J* = 4.9 Hz, 2H), 2.42 (d, *J* = 5.7 Hz, 2H), 2.39 (s, 3H), 1.63 (t, *J* = 3.7 Hz, 4H); ^{13}C NMR (101 MHz, Methanol-d_4) δ 167.15, 163.31, 157.93, 157.61, 139.97, 139.11, 138.69, 131.92, 131.46, 130.32, 130.30, 127.19, 121.65, 118.86, 117.23, 110.22, 110.00, 105.56, 88.74, 68.86, 65.82, 54.72, 51.98, 34.12, 31.44, 28.27, 28.04, 17.77; ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.83, 161.91, 158.76, 157.50, 140.51, 139.44, 139.20, 132.41, 131.55, 130.78, 130.74, 127.51, 122.53, 119.25, 117.96, 110.82, 109.62, 105.91, 88.69, 68.15, 66.70, 54.97, 52.27, 34.47, 31.62, 28.88, 28.57, 20.37.

For **29b**: LCMS (ESI) *m/z* 528 $[M+H]^+$; t_R: 1.77 min; HRMS calcd for $C_{29}H_{34}N_7O_3$ (MH^+) 528.2723, found 528.2718; ^{1}H NMR (300MHz, DMSO-d_6): δ 7.94 (s, 1H), 7.83 (s, 1H), 7.77 (s, 1H), 7.51-7.38 (m, 3H), 6.89 (s, 1H), 6.74 (d, *J*=6.9 Hz, 1H), 6.27 (s, 1H), 5.88 (s, 1H), 5.68 (s, 2H), 5.47-5.31 (m, 4H), 5.17 (s, 2H), 4.19 (br, 2H), 3.88-3.84 (m, 4H), 2.37-2.28 (m, 5H), 2.08-1.99 (m, 2H), 1.79-1.70 (m, 2H).

Scheme 6. Synthesis of Macrocycle 30[a]



[a]Reagents and conditions: (a) HATU, DIPEA, DM, rt, 12 h, 37%; (b) Grubbs' catalyst (II), DCE, 70 °C, 12 h, 15%.

1-(4-((1H-pyrazol-1-yl)methyl)-2-(but-3-enyloxy)benzyl)-N-((6-amino-2-methyl-4-(pent-4-enyloxy)pyridin-3-yl)methyl)-1H-pyrazole-4-carboxamide (6). The title compound was prepared from acid **7** and amine **10** according to the general procedure of amide coupling with HATU (Method A) (yellow solid, 37%). LCMS (ESI) *m/z* 556 $[M+H]^+$.

20-Amino-22-methyl-6-(1H-pyrazol-1-ylmethyl)-9,17-dioxa-1,21,25,29-tetraazatetracyclo[25.2.1.0^{3},^{8}.0^{18},^{23}]triaconta-3,5,7,12,18(23),19,21,27(30),28-nonaen-26-one (cmpd30). The title compound was prepared from **6** according to the general procedure of Grubbs RCM (Method B). The product (41.7 mg, 15 %) was obtained as a gray solid (mixture of cis- and transisomers). LCMS (ESI) *m/z* 528 $[M+H]^+$; HRMS calcd for $C_{29}H_{34}N_7O_3$ (MH^+) 528.2723, found 528.2731; ^{1}H NMR (300 MHz, DMSO- *d*6) δ 7.94 (s, 1H), 7.84 (s, 1H), 7.75-7.67 (m, 2H), 7.45-7.34 (m, 2H), 6.87-6.84 (m, 1H), 6.75 (d, *J* =7.5 Hz, 1H), 6.26 (s, 1H), 6.03 (s, 1H), 5.36-5.28 (m, 3H), 5.14-5.06 (m, 3H), 4.20 (s, 2H), 3.96-3.78 (m, 4H), 2.42-2.18 (m, 5H), 1.93-1.79 (m, 2H), 1.65-1.64 (m, 2H).

Crystallography: Proteins used for the studies contain the catalytic protease domain (PD) of pKal, containing three deglycosylation mutations (N396E, N453E, and N494E) as well as two cysteine mutations (C383S and C503S) was recombinantly expressed in insect cells and purified by Beryllium (Bedford, MA).

For crystallography screening we used a mosquito liquid handling robot (TTP Lab Tech) and three commercially available deep well screening blocks: JCSG+ and Protein Complex Suite (Qiagen) as well as the Index HT screen (Hampton Research). Initial crystallization conditions for the pKal PD protein were based of the manuscript detailing the structure of pKal bound to benzamidine (0.2 M potassium di-hydrogen phosphate, 20% (v/v) PEG3350. Using a protein concentration of 15-20 mg/ml the final crystallization condition for pKal PD was refined to 0.2 M potassium di-hydrogen phosphate, 23% (v/v) PEG3350 using the hanging drop vapor diffusion method at 23 °C. Crystals were transferred to solution containing the same mother liquor plus 2 mM concentration of compound at 23 ℃. Crystals were cryoprotected by adding glycerol (10-12% (v/v) final concentration) to the reservoir solution containing 2 mM compound before flash-freezing in liquid nitrogen. Crystal screening and final data collection was carried out at beamline 8.3.1 at the Advanced Light Source (ALS).

Structure Determination: Data reduction was carried out using iMOSFLM and the CCP4 software suite (Battye et al., 2011; Winn et al., 2011). Data was solved using molecular replacement methods in the PHENIX program suite (Adams et al., 2010). The structures were solved with molecular replacement using the structure of human plasma kallikrein bound to benzamidine (PDB code 2ANW)

(Tang, 2005). All models were built using COOT and further refinement was carried out using the latest builds of the PHENIX suite (Adams et al., 2010) Supplemental Table below. A majority of the secondary structural elements have been built. Some loops have been omitted due to poor electron density and very high B-factors. The final model has been deposited with the RCSB and the assigned the PDB code.[1] Figures were made using PyMOL (Schrodinger, 2016).

pKal protease domain macrocycle **32**	
Data Collection	
Wavelength	1.11
Space group	$P2_12_12_1$
Cell dimensions:	
a,b,c (Å)	55.66, 59.62, 77.11
β (°)	90.00
Resolution (Å)	40.0-1.4 (1.46-1.41)
R_{sym}[a]	0.057 (0.372)
$R_{p.i.m.}$	0.031 (0.194)
Completeness	97.2 (95.8)
Redundancy	4.3 (4.2)
I/σ	13.2 (3.8)
Wilson B factor (Å^2)	12.86
Refinement	
Resolution (Å)	27.8-1.4
Reflections	48,771
Nonhydrogen Atoms	2210
Water Molecules	368
$R_{\mathbf{work}}$[b]	16.25
$R_{\mathbf{free}}$[c]	18.6
R.m.s. deviations	
Bond lengths (Å)	0.006
Bond angles (°)	1.067
B factors (Å^2)	
Protein	17.2
Ligands	14.8
Water	31.9
Coordinate error (Å)	0.11
Ramachandran plot[d]	
Most favored (%)	98
Allowed (%)	2
Disallowed (%)	0

Highest resolution shell is shown in parenthesis.

[a] $R_{sym} = \sum|I_i - \langle I_i \rangle| / \sum I_i$, where I_i is the intensity of the *i*th observation and $\langle I_i \rangle$ is the mean intensity of the reflection.

[b] $R_{p.i.m.} = \sum_{hkl}[1/(N-1)]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity and $\langle I(hkl) \rangle$ is the average intensity of multiple observations of symmetry-related reflections.

[c] $R_{work} = \sum(||F_{obs}| - |F_{calc}|| / \sum|F_{obs}|)$

[d] R_{free} = R value for a randomly selected subset (5%) of the data that were not used for minimization of the crystallographic residual.

[e] Calculated with the program PROCHECK (32).

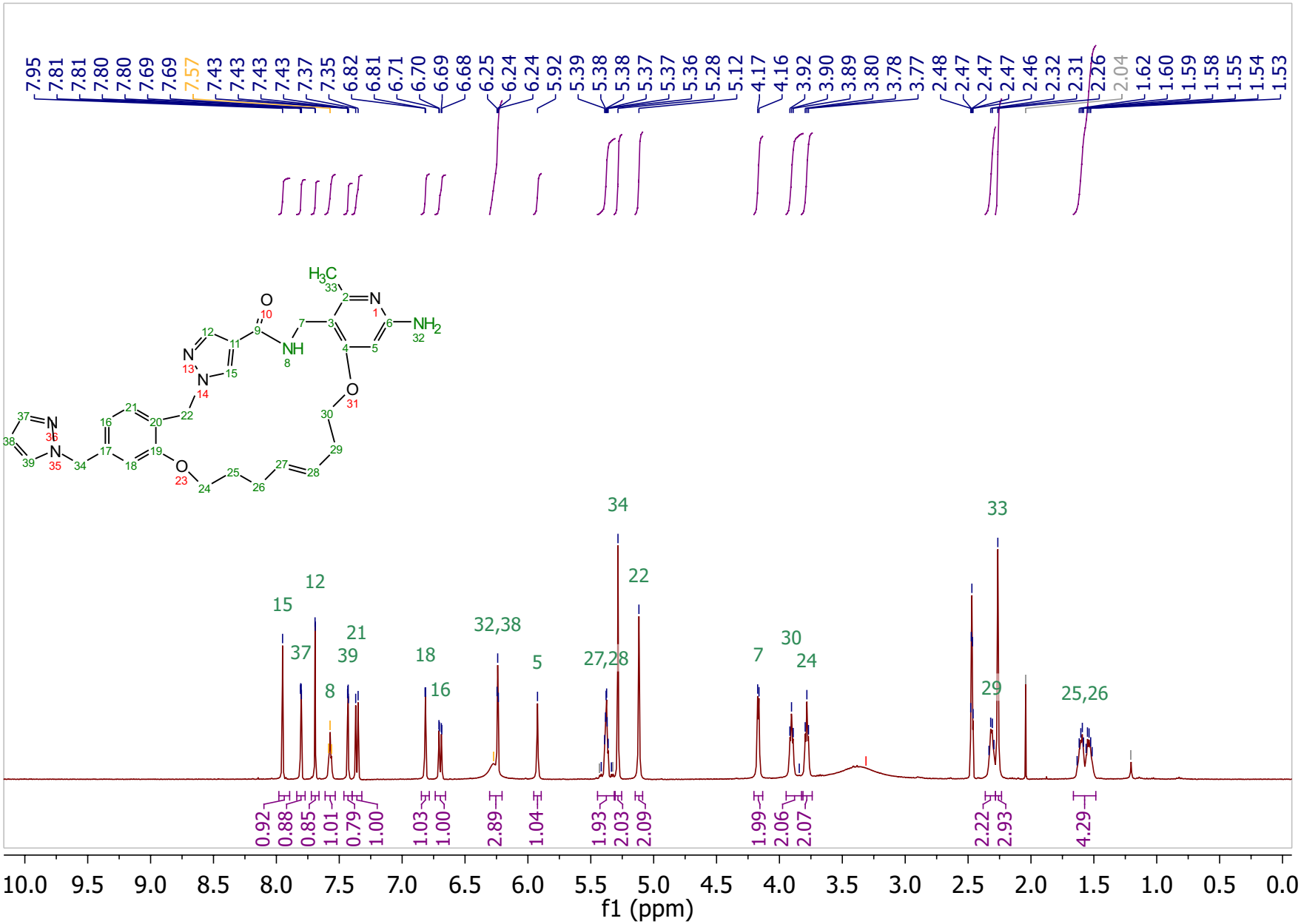
Molecular Modeling. MOE (Molecular Operating Environment)[2] from the Chemical Computing Group (Montreal, Canada) was used for this work. Structure of the active domain of plasma kallikrein (PDB ID: 5TJX) was prepared by using the LigX module in MOE2014. Default program settings and Amber10 EHT force field were applied. The prepared structure was used for all modeling and design work.

References

1) Partridge, J.; Choy, R., Li, Z. Manuscript in preparation.

2) *Molecular Operating Environment (MOE)*, 2014.09; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, **2014**.

Compound 29a (cis- or trans- isomer)



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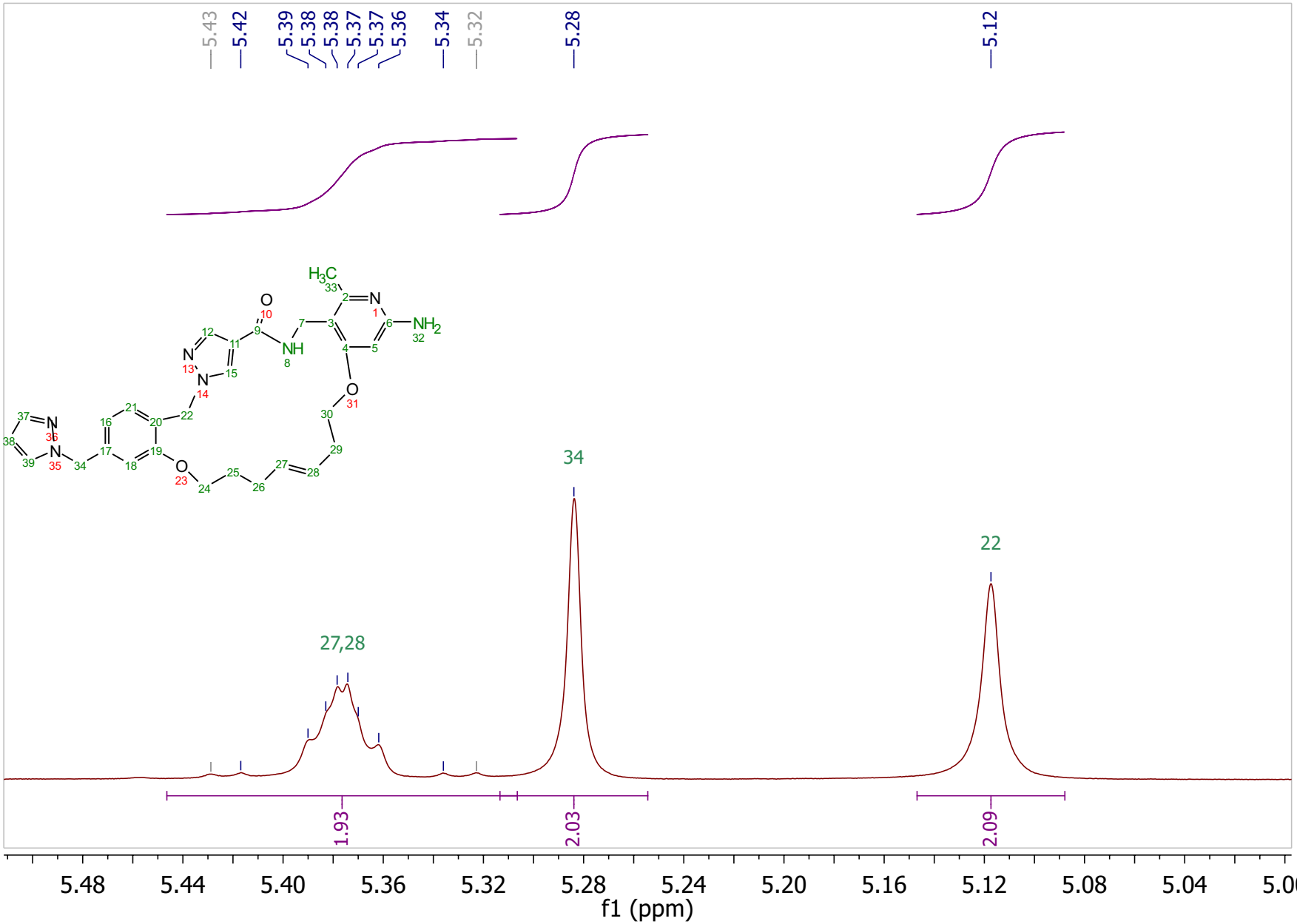
Acquisition Time:	2.5559s
Acquisition Date:	2016-11-08T14:53:15
Spectrom. Freq.:	399.8MHz
Spectral Width:	6410.3
Nucleus:	1H
Acquired Size:	16384
Spectral Size:	65536
DC:	0.05
Apodization:	
FT:	Hyper Invert Quadrature
Phase:	Global
Baseline Correction:	Bernstein, order =3

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.80 (dd, J = 2.2, 0.7 Hz, 1H), 7.69 (d, J = 0.7 Hz, 1H), 7.57 (t, J = 4.3 Hz, 1H), 7.43 (dd, J = 1.8, 0.7 Hz, 1H), 7.36 (d, J = 7.6 Hz, 1H), 6.82 (d, J = 1.5 Hz, 1H), 6.70 (dd, J = 7.6, 1.4 Hz, 1H), 6.30 – 6.21 (m, 3H), 5.92 (s, 1H), 5.38 (td, J = 4.8, 3.1 Hz, 2H), 5.28 (s, 2H), 5.12 (s, 2H), 4.17 (d, J = 4.2 Hz, 2H), 3.90 (t, J = 5.3 Hz, 2H), 3.78 (t, J = 5.2 Hz, 2H), 2.31 (q, J = 5.2 Hz, 2H), 2.26 (s, 3H), 1.57 (ddt, J = 27.2, 9.7, 5.6 Hz, 4H).

Compound 29a (cis- or trans- isomer)



Parameter	Value
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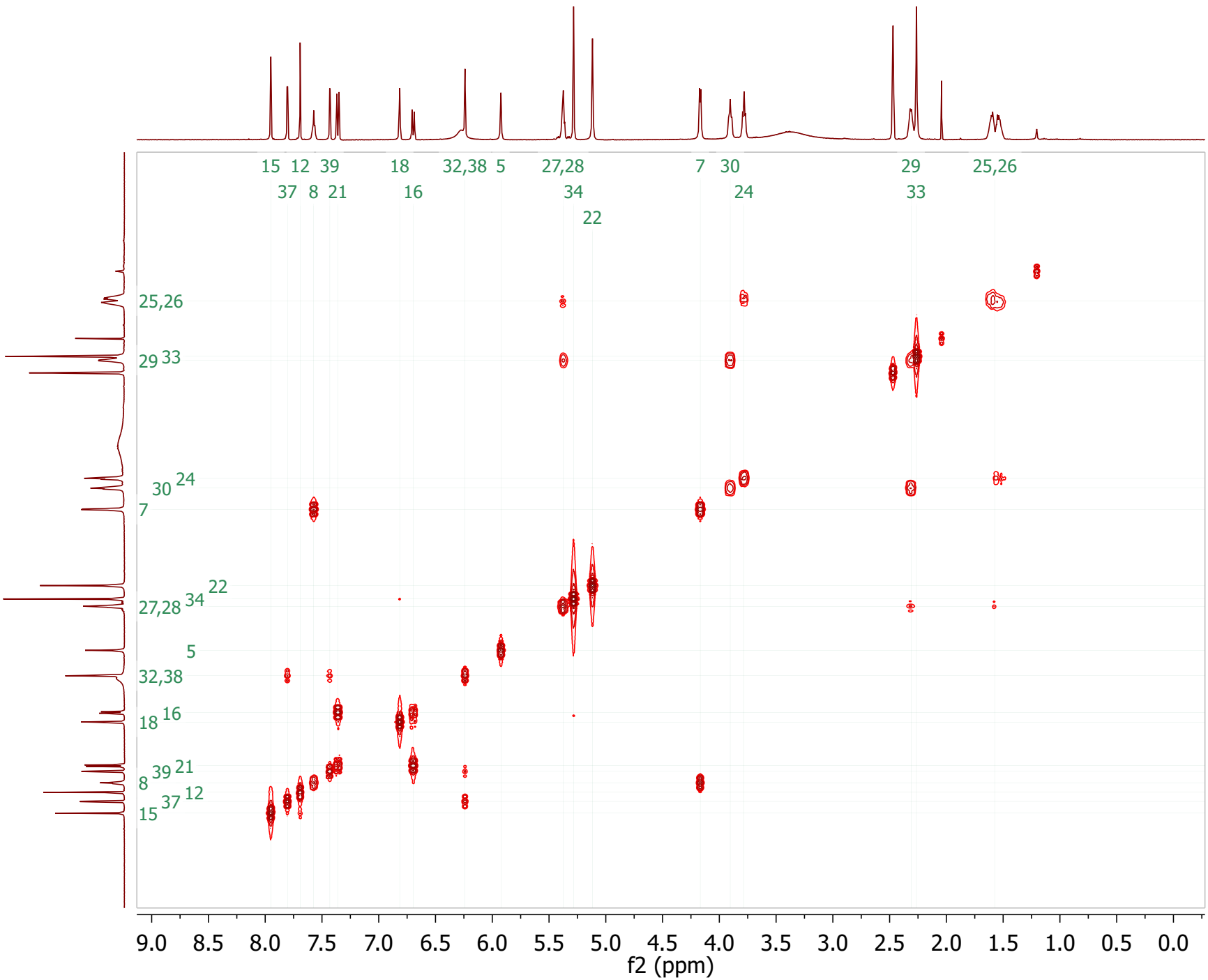
Relaxation Delay:	1s
Acquisition Time:	2.5559s
Acquisition Date:	2016-11-08T14:53:15
Spectrom. Freq.:	399.8MHz
Spectral Width:	6410.3
Nucleus:	1H
Acquired Size:	16384
Spectral Size:	65536
DC:	0.05
Apodization:	
FT:	Hyper Invert Quadrature
Phase:	Global
Baseline Correction:	Bernstein, order =3

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.80 (dd, J = 2.2, 0.7 Hz, 1H), 7.69 (d, J = 0.7 Hz, 1H), 7.57 (t, J = 4.3 Hz, 1H), 7.43 (dd, J = 1.8, 0.7 Hz, 1H), 7.36 (d, J = 7.6 Hz, 1H), 6.82 (d, J = 1.5 Hz, 1H), 6.70 (dd, J = 7.6, 1.4 Hz, 1H), 6.30 – 6.21 (m, 3H), 5.92 (s, 1H), 5.38 (td, J = 4.8, 3.1 Hz, 2H), 5.28 (s, 2H), 5.12 (s, 2H), 4.17 (d, J = 4.2 Hz, 2H), 3.90 (t, J = 5.3 Hz, 2H), 3.78 (t, J = 5.2 Hz, 2H), 2.31 (q, J = 5.2 Hz, 2H), 2.26 (s, 3H), 1.57 (ddt, J = 27.2, 9.7, 5.6 Hz, 4H).

Compound 29a (cis- or trans- isomer) gCOSY



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Spectral Width:	[3765.1, 3765.1]
Nucleus:	[1H, 1H]
Acquired Size:	[256, 565]

F2 Processing

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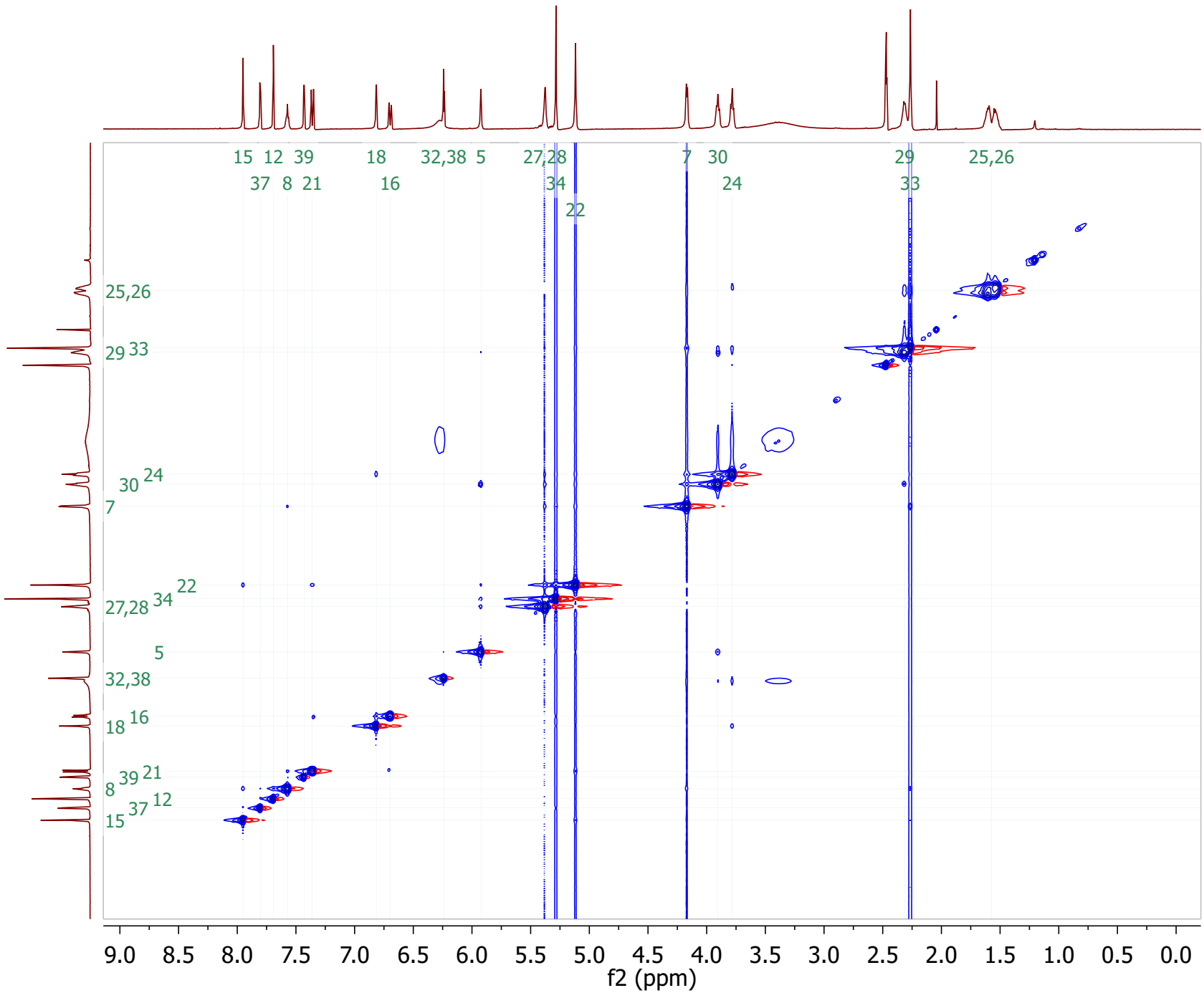
Apodization:	
FT:	Hyper Invert Quadrature
Phase:	Magnitude
Baseline Correction:	None

F1 Processing

SI:	1024
Apodization:	
FT:	Hyper Invert Quadrature

Compound 29a (cis- or trans- isomer) NOESY_01



Parameter	Value [F1,F2]
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Spectral Width:	[3742.5, 3742.5]
Nucleus:	[1H, 1H]
Acquired Size:	[256, 561]

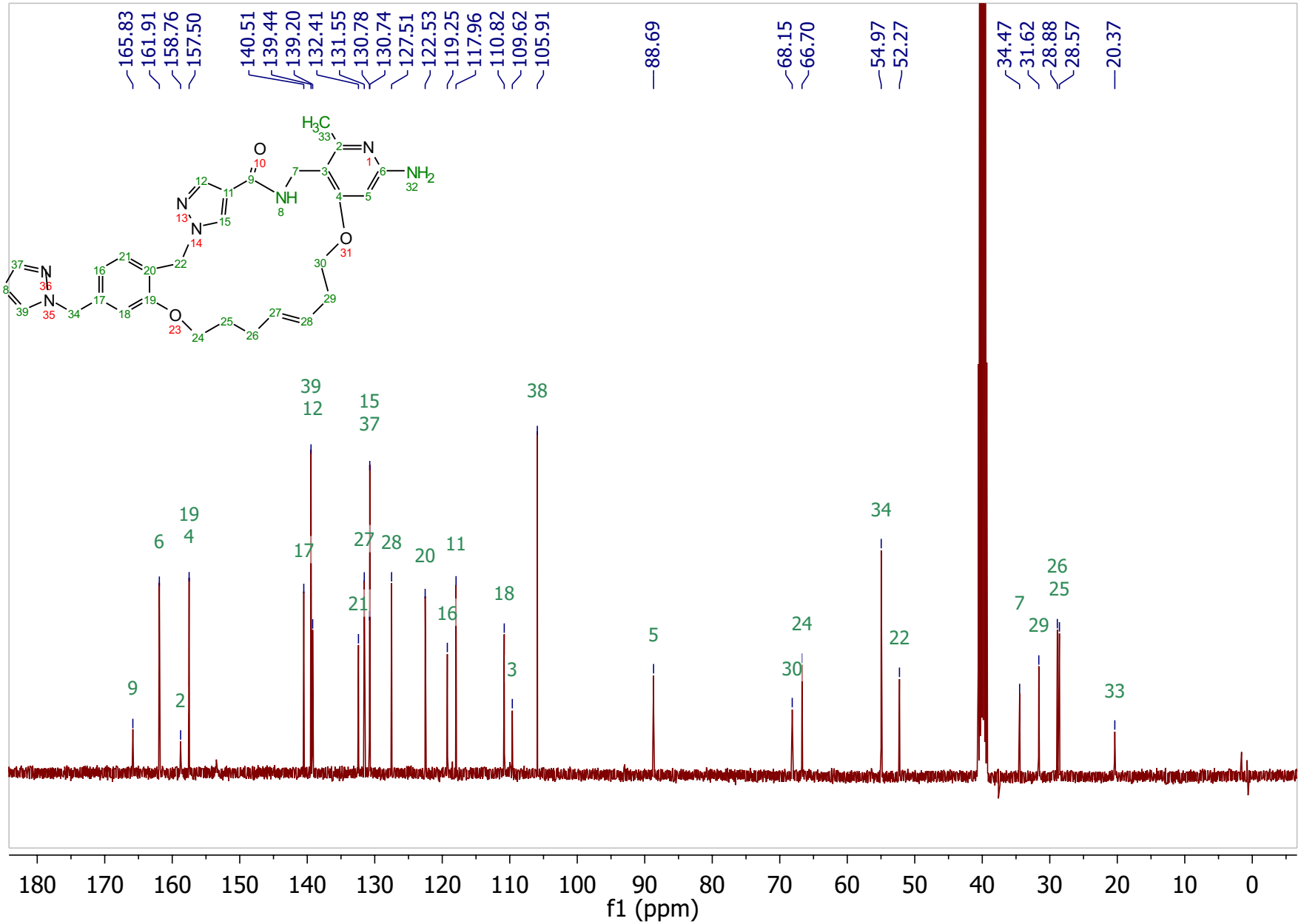
F2 Processing

Spectral Size:	1024
DC:	0.05
Apodization:	Gauss: 7.65 First Point: 0.50
FT:	Hyper Invert Quadrature
Phase:	Imported: Ph0: 20.08, Ph1: 0.00
Baseline Correction:	None

F1 Processing

SI:	1024
Apodization:	Gauss: 14.39
FT:	Hyper Hyper Invert

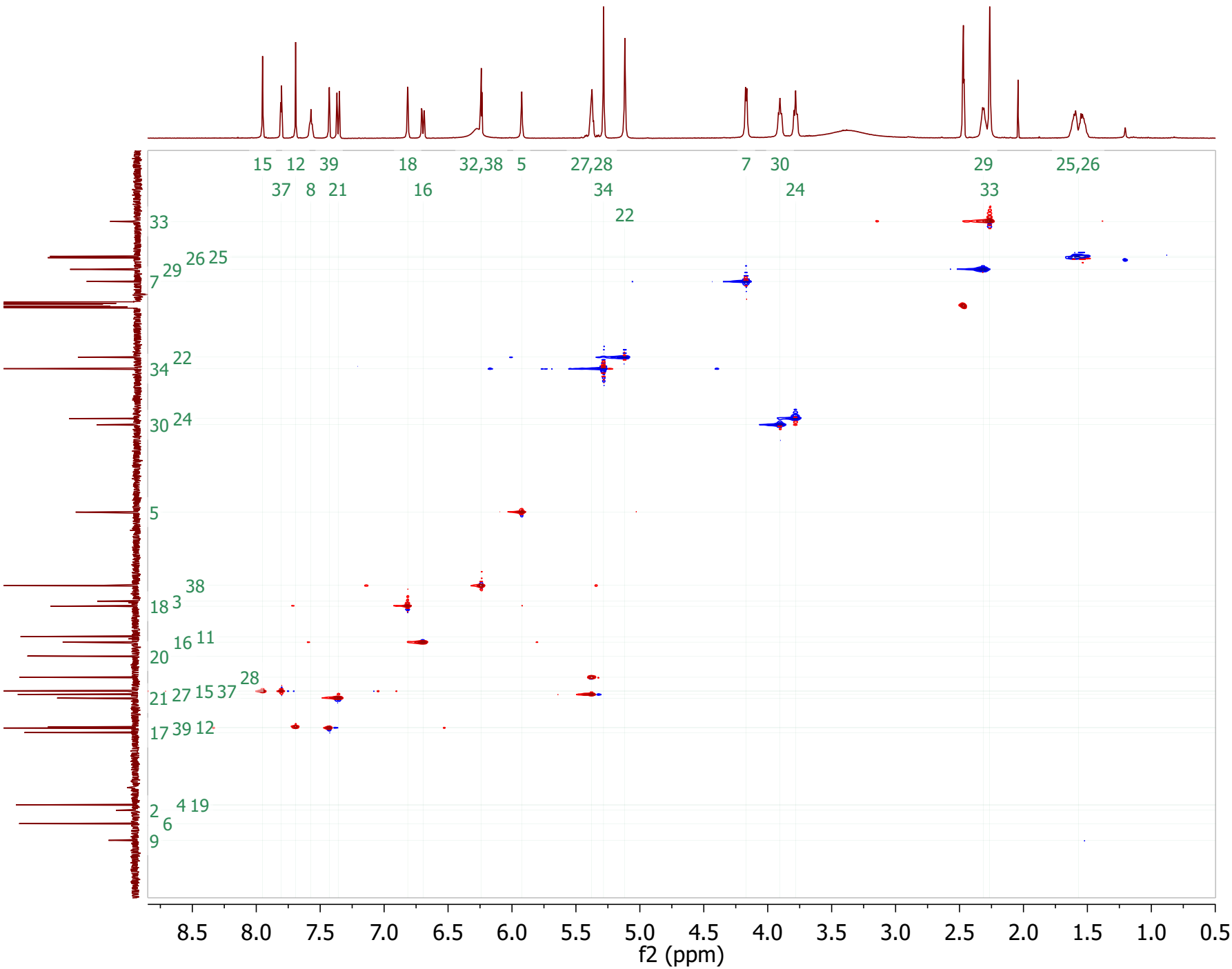
Compound 29a (cis- or trans- isomer) CARBON-13



Parameter	Value
Title:	CARBON_01
Data File Name:	I:/NMR/compound 29a/CARBON_01.fid/fid
Comment:	
Spectrometer:	vnmrs
Solvent:	dmso
Temperature:	25
Number of Scans:	10000
Relaxation Delay:	1s
Acquisition Time:	1.3107s
Acquisition Date:	2016-11-08T18:01:57
Spectrom. Freq.:	100.54MHz
Spectral Width:	25000
Nucleus:	13C
Acquired Size:	32768
Spectral Size:	65536
DC:	0.05
Apodization:	LB: 0.50 Hz
FT:	Hyper Invert Quadrature
Phase:	Imported: Ph0: -175.09, Ph1: 0.00
Baseline Correction:	None

^{13}C NMR (101 MHz, dmso) δ 165.83, 161.91, 158.76, 157.50, 140.51, 139.44, 139.20, 132.41, 131.55, 130.78, 130.74, 127.51, 122.53, 119.25, 117.96, 110.82, 109.62, 105.91, 88.69, 68.15, 66.70, 54.97, 52.27, 34.47, 31.62, 28.88, 28.57, 20.37.

Compound 29a (cis- or trans- isomer) HSQCAD_01



Parameter	Value [F1,F2]
Title:	HSQCAD_01
Data File Name:	I:/NMR/compound 29a/HSQCAD_01.fid/fid
Comment:	
Spectrometer:	vnmrs
Solvent:	dmso
Temperature:	25
Number of Scans:	8
Relaxation Delay:	1s
Acquisition Time:	0.1501s
Acquisition Date:	2016-11-09T00:27:16
Spectrom. Freq.:	[100.54, 399.80]MHz
Spectral Width:	[20105.6, 6410.3]
Nucleus:	[13C, 1H]
Acquired Size:	[512, 962]

F2 Processing

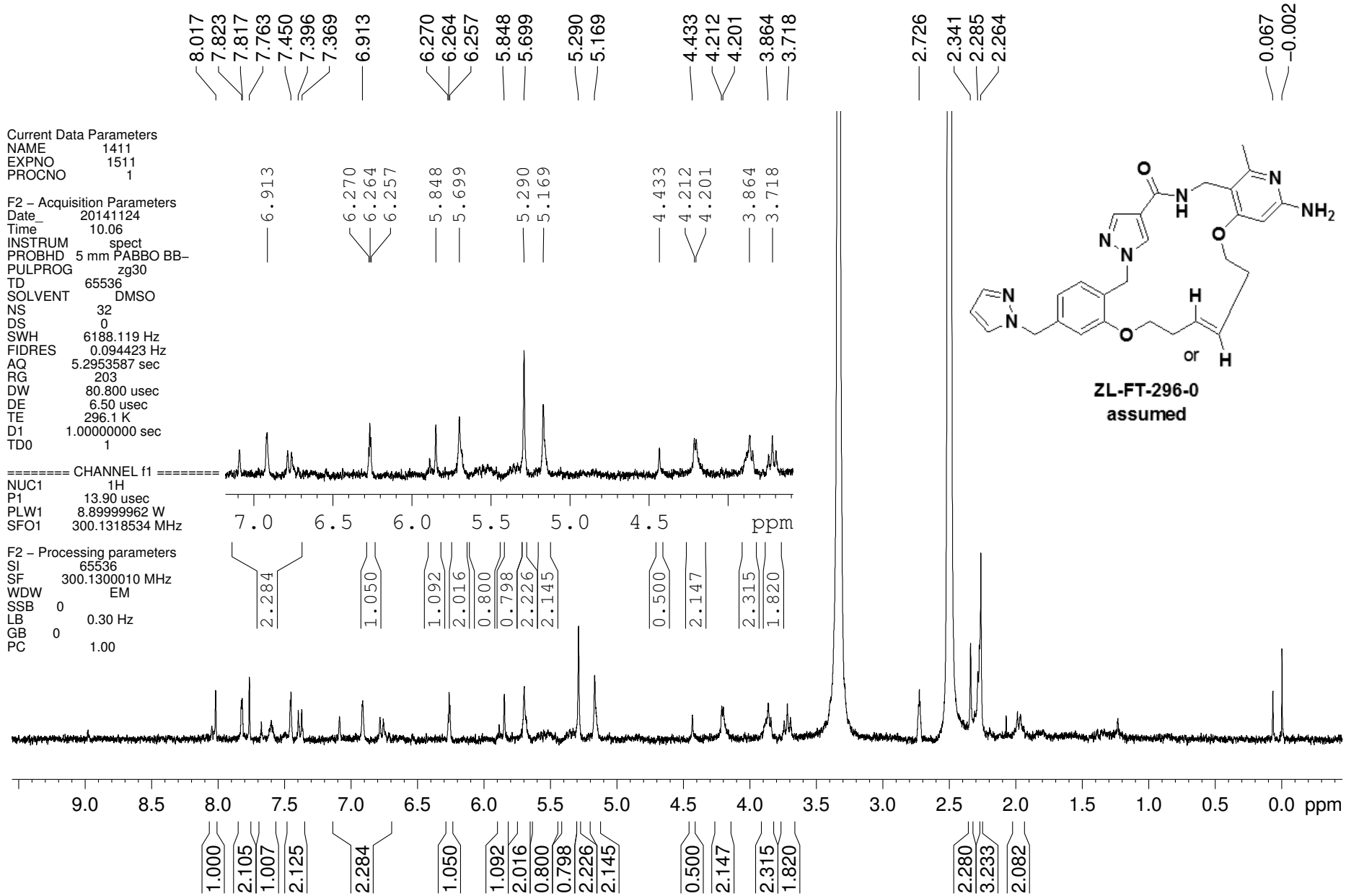
Spectral Size:	1024
DC:	0.05
Apodization:	Gauss: 7.64
FT:	Hyper Invert Quadrature
Phase:	Imported: Ph0: 20.08, Ph1: 0.00
Baseline Correction:	None

F1 Processing

SI:	1024
Apodization:	Gauss: 22.55

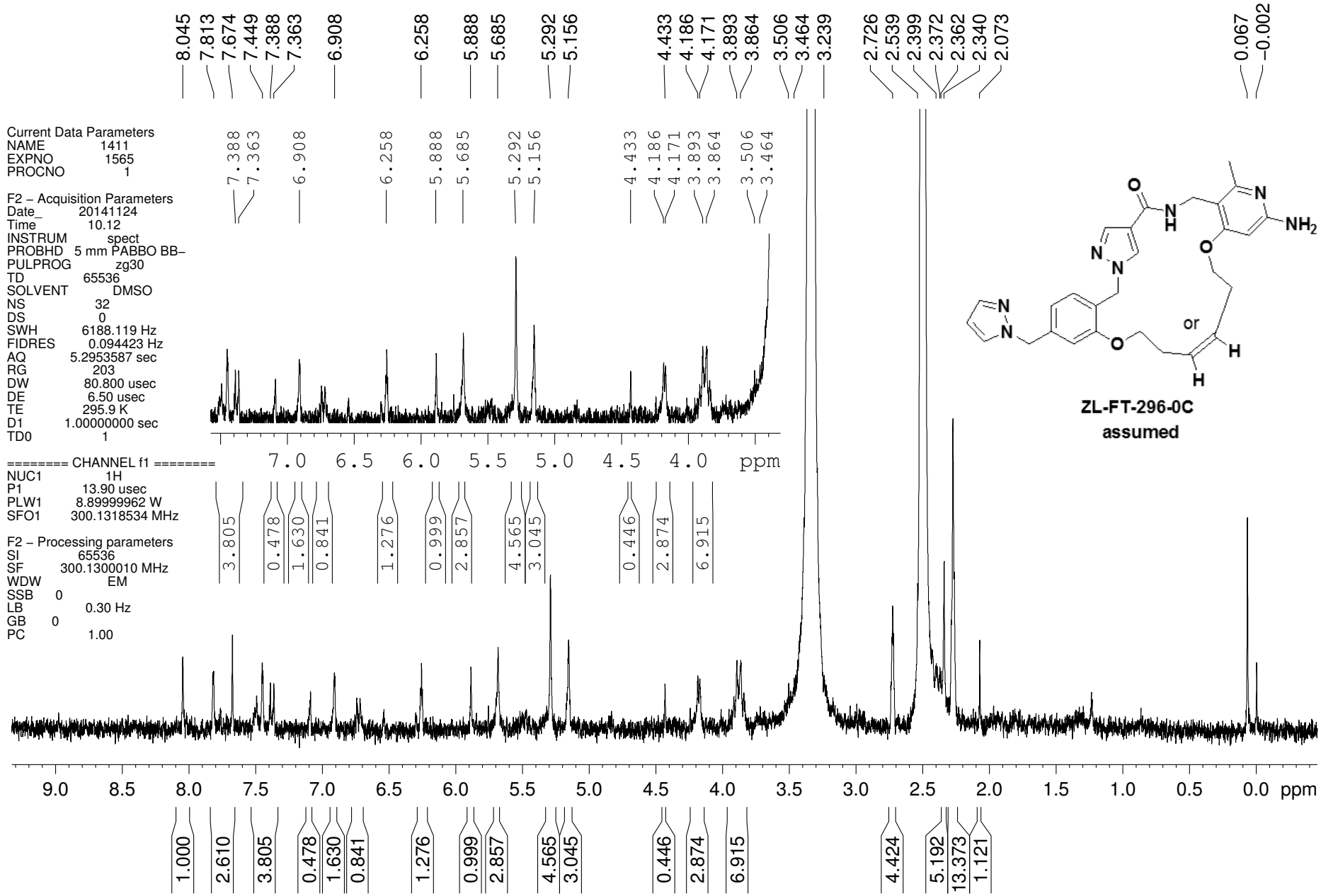
Cmpd3a

H–NMR04–PH–GBT–ZL–FT–296–0–1(53122–016A1)1T,2014111511
DMSO



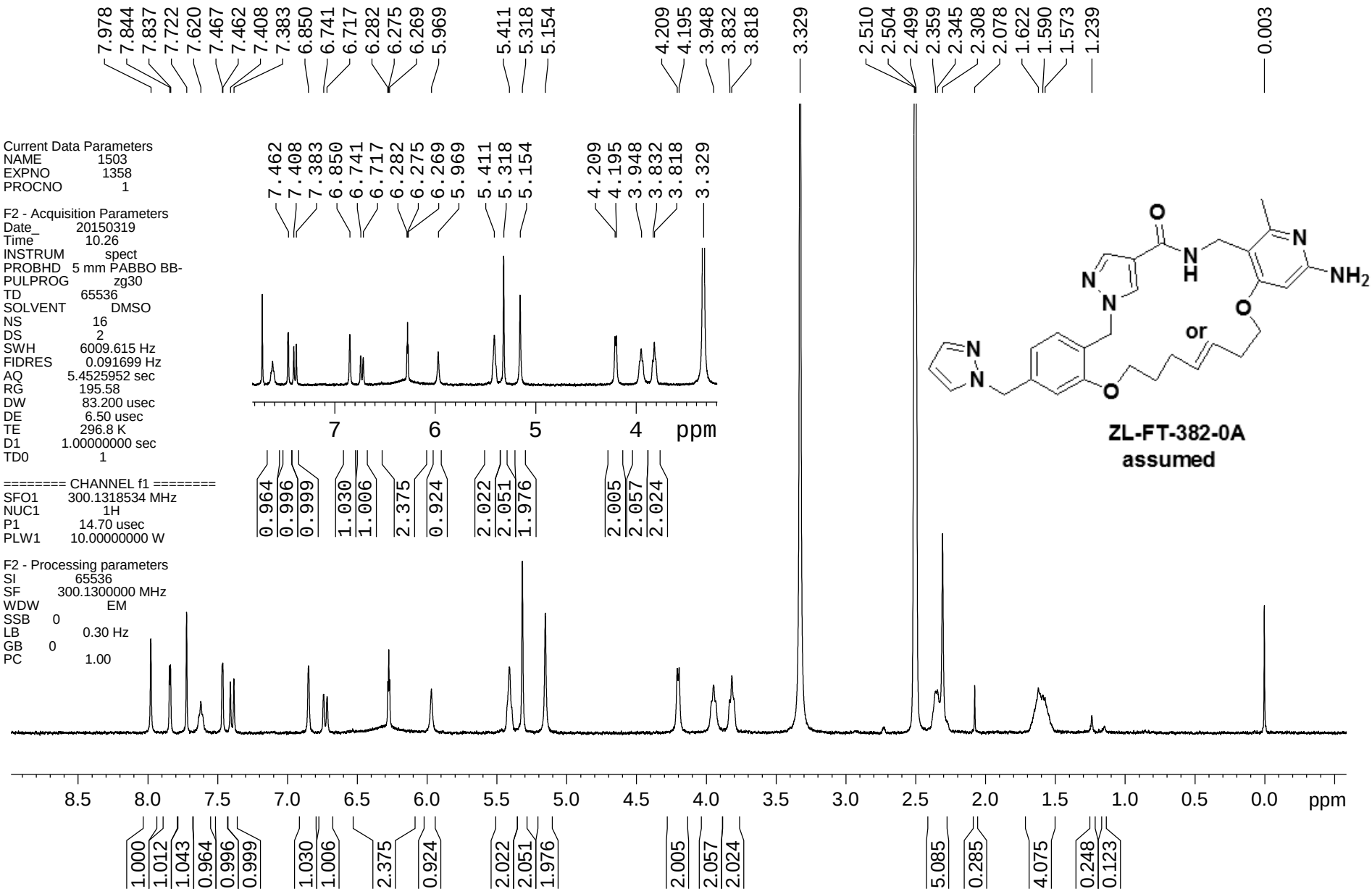
Cmpd3b

H–NMR04–PH–GBT–ZL–FT–296–0C–1(53122–017A1)1T,2014111565
DMSO



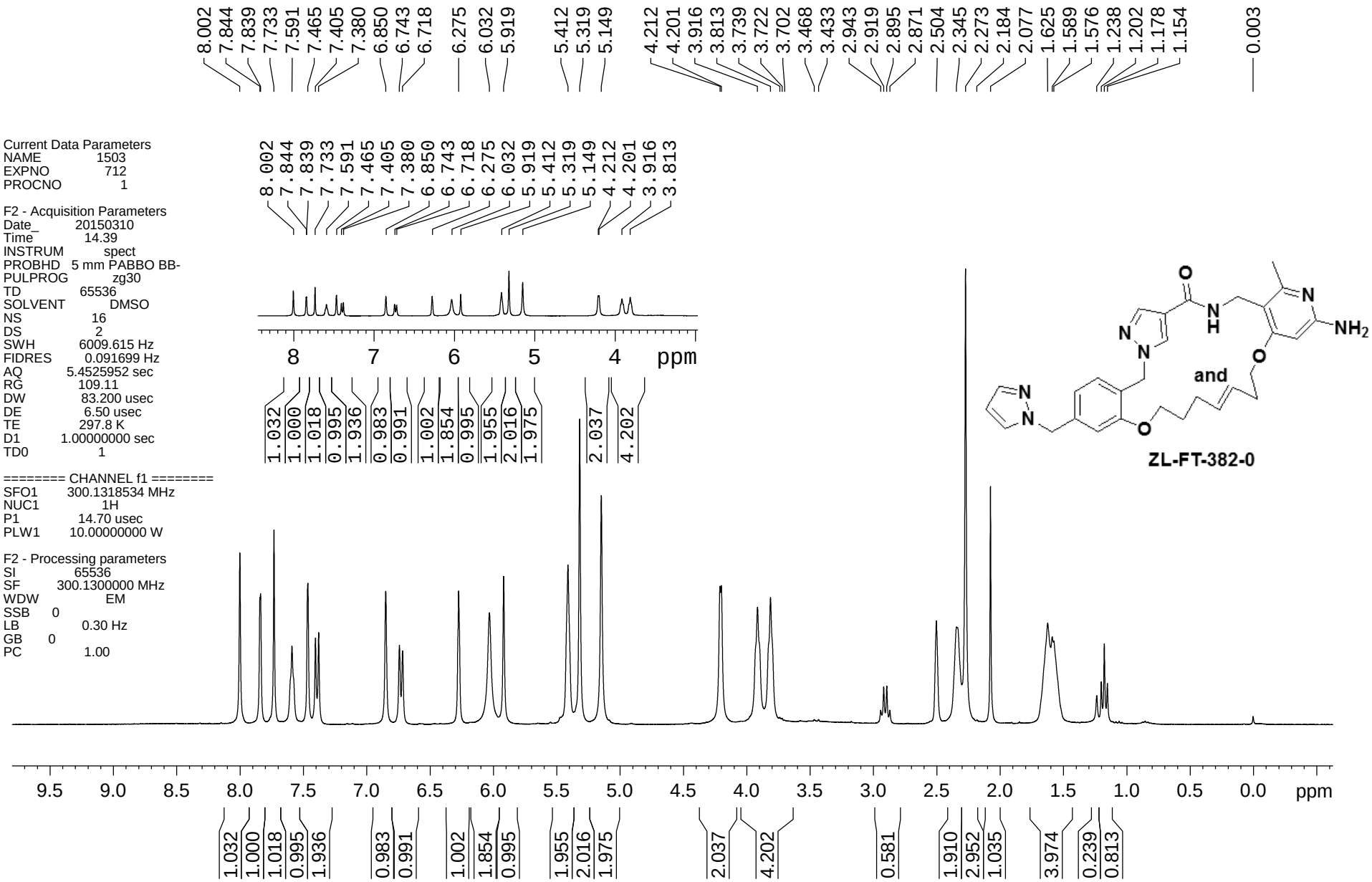
Cmpd29a

H-NMR09-PH-GBT-ZL-FT-382-0A-3(53134-139E1)1T,H2015031358
DMSO



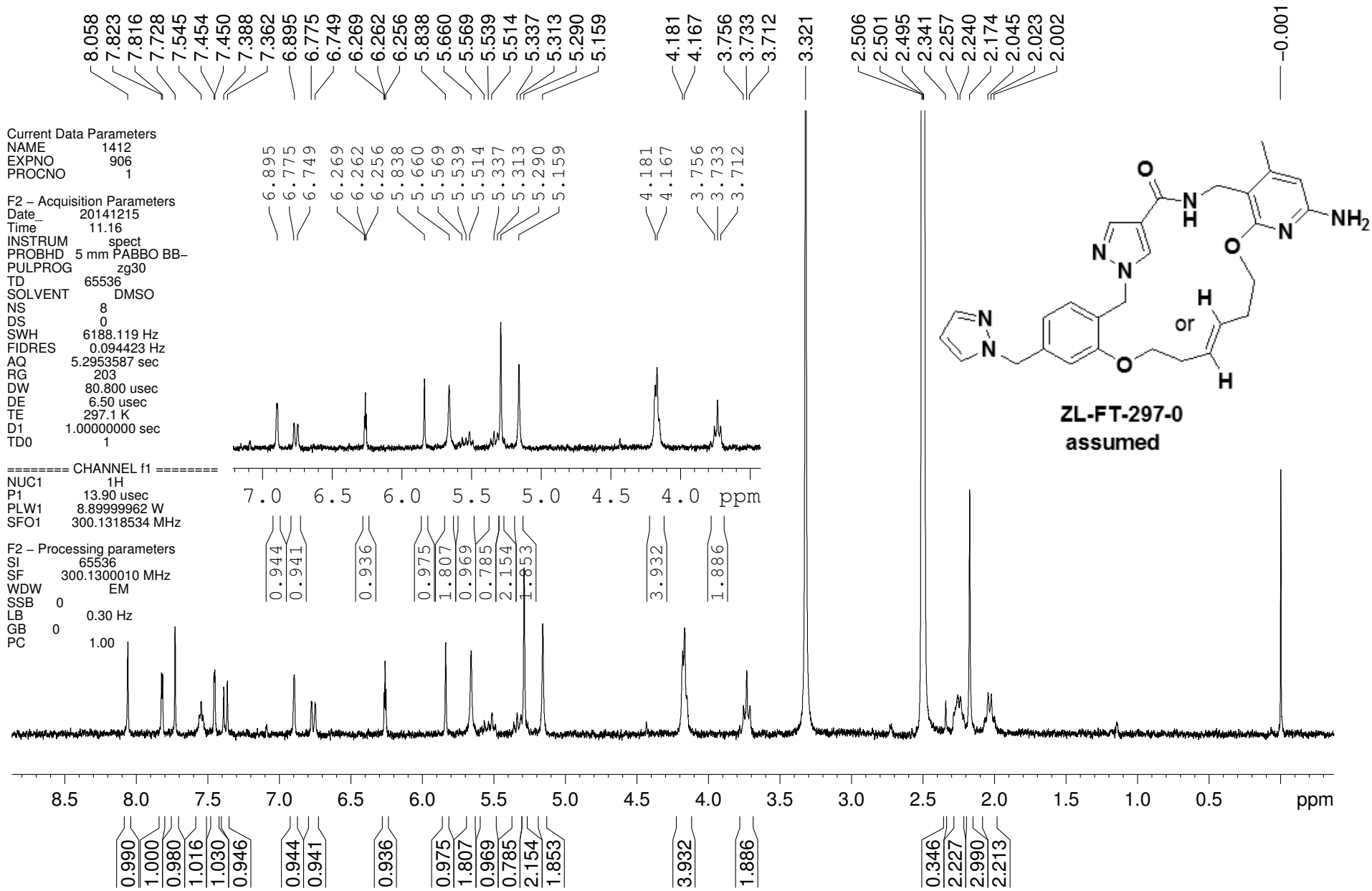
Cmpd29b

H-NMR09-PH-GBT-ZL-FT-382-0-3(53134-139E1)1T,H2015030712
DMSO



Cmpd32a

H–NMR04–PH–GBT–ZL–FT–297–0–1(53122–019A1)1T,2014120906
DMSO



Cmpd32b

H–NMR04–PH–GBT–ZL–FT–297–0A–1(53122–018A1)1T,2014120905
DMSO